



AGRICULTURAL RESEARCH INSTITUTE

PUSA

JOURNAL OF AGRICULTURAL RESEARCH

VOLUME 45

JULY 1-DECEMBER 15, 1932



ISSUED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE
WITH THE COOPERATION OF THE ASSOCIATION OF
LAND-GRANT COLLEGES AND UNIVERSITIES

UNITED STATES
GOVERNMENT PRINTING OFFICE
WASHINGTON : 1933

JOINT COMMITTEE ON POLICY AND MANUSCRIPTS

FOR THE UNITED STATES DEPARTMENT OF AGRICULTURE

H. G. KNIGHT, CHAIRMAN
Chief, Bureau of Chemistry and Soils

F. L. CAMPBELL
Entomologist, Bureau of Entomology

JOHN W. ROBERTS
*Senior Pathologist, Bureau of Plant
Industry*

FOR THE ASSOCIATION OF LAND-GRANT COLLEGES AND UNIVERSITIES

S. W. FLETCHER
*Director of Research, Pennsylvania Agri-
cultural Experiment Station*

S. B. DOTEN
*Director, Nevada Agricultural Experiment
Station*

C. G. WILLIAMS
*Director, Ohio Agricultural Experiment
Station*

EDITORIAL SUPERVISION

M. C. MERRILL

Chief of Publications, United States Department of Agriculture

Articles for publication in the Journal must bear the formal approval of the chief of the department bureau or of the director of the experiment station from which the paper emanates. Each manuscript must be accompanied by a statement that it has been read and approved by one or more persons (named) familiar with the subject. The data as represented by tables, graphs, summaries, and conclusions must be approved from the statistical viewpoint by someone (named) competent to judge. All computations should be verified.

Station manuscripts and correspondence concerning them should be addressed to S. W. Fletcher, Director of Research, Pennsylvania Agricultural Experiment Station, State College, Pa.

Published on the first and fifteenth of each month. This volume will consist of twelve numbers and the Contents and Index.

Subscription price:

Entire Journal: Domestic, \$2.25 a year (2 volumes)

Foreign, \$3.50 a year (2 volumes)

Single numbers: Domestic, 10 cents

Foreign, 15 cents

Articles appearing in the Journal are printed separately and can be obtained by purchase at 5 cents a copy domestic; 8 cents foreign. If separates are desired in quantity, they should be ordered at the time the manuscript is sent to the printer. Address all correspondence regarding subscriptions and purchase of numbers and separates to the Superintendent of Documents, Government Printing Office, Washington, D. C.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 45

WASHINGTON, D. C.,

JULY 1-DECEMBER 15, 1932

CONTENTS

	Page
A Classification of the Varieties of Field Beans, <i>Phaseolus vulgaris</i> : F. H. STEINMETZ and A. C. ARNY. Illus.	1
The Influence of Certain Balanced Rations on the Chemical and Physical Properties of Milk Fat: O. R. OVERMAN and O. F. GARRETT	51
The Migration of <i>Bacillus amyglororus</i> in the Tissue of the Quince: HERBERT A. WAHL. Illus.	59
Some Physiological Studies of <i>Gloeosporium perennans</i> and <i>Neofabraea malicorticis</i> : ERSTON V. MILLER. Illus.	65
<i>Macrocentrus ancyliorvus</i> Roh., An Important Parasite of the Oriental Fruit Moth: G. J. HAEUSSLER. Illus.	79
Effect of Size of Crown and Length of Cutting Season on Yields of Asparagus: E. S. HABER	101
The Value of Iodine for Livestock in Central Pennsylvania: E. B. FORBES, GEO. M. KARNS, S. I. BECHDEL, P. S. WILLIAMS, T. B. KEITH, E. W. CALLENBACH, and R. R. MURPHY	111
The Role of Nitrogen in the Production of Spots in Wheat Fields: P. L. GAINEY and M. C. SEWELL. Illus.	129
Nitrogen Changes Produced in Certain Nitrogenous Compounds by Azotobacter and the Nitrogen Fixed in the Presence of These Compounds: L. G. THOMPSON, JR.	149
The Effect of the Amount of Feed Consumed by Cattle on the Utilization of Its Energy Content: H. H. MITCHELL, T. S. HAMILTON, F. J. McCLEURE, W. T. HAINES, JESSIE R. BEADLES, and H. P. MORRIS. Illus.	163
<i>Sturmin inconspicuus</i> Meigen, a Tachinid Parasite of the Gipsy Moth: R. T. WEBBER. Illus.	193
Correlational and Allied Studies of the Protein Content, Water Absorption, Loaf Volume, and Loaf Weight of Two Series of Hard Red Spring Wheats: L. R. WALDRON and C. E. MANGELS. Illus.	209
Some Minor Strains of Southern Pine and Hardwood Lumber and Logs: T. C. SCHEFFER and R. M. LINDGREN	233
The Relation of Agronomic Practice to the Quantity and Quality of the Oil in Flaxseed: I. J. JOHNSON. Illus.	239
Relation of Commercial Honey to the Spread of American Foulbrood: A. P. STURTEVANT. Illus.	257
Heterothallism and Hybridization in <i>Sphaerellotheca sorghi</i> and <i>S. eruenta</i> : H. A. RODENHUSER. Illus.	287
Physiologic Specialization in <i>Puccinia graminis secalis</i> : RALPH U. COTTER and MOSES N. LEVINE. Illus.	297
Lead Arsenate Poisoning in Chickens: E. F. THOMAS and A. L. SHEALY	317
Rose Anthracnose Caused by <i>Sphaeloma</i> : ANNA E. JENKINS. Illus.	321
A Bacterial Disease of the Tung-Oil Tree: LUCIA McCULLOCH and J. B. DEMAREE. Illus.	339
Detecting Pink Bollworms in Cottonseeds by the X Ray: F. A. FENTON and WILLIS W. WAITE. Illus.	347
The Effect of the Degree of Slope on Run-Off and Soil Erosion: F. L. DULEY and O. E. HAYS. Illus.	349
Deterioration in Shelled Green Peas Held a Few Days in Storage Prior to Canning: Z. I. KERTESZ and E. L. GREEN	361
Life History and Habits of Crested Wheatgrass: L. DUDLEY LOVE and HERBERT C. HANSON. Illus.	371
Quantity of Milk Obtained from Amputated Cow Udders: W. W. SWETT, FRED W. MILLER, and R. R. GRAVES. Illus.	385
Composition of Milk Obtained from Amputated Cow Udders: W. W. SWETT, FRED W. MILLER, and R. R. GRAVES. Illus.	401
The Origin, Development, and Increase of Chloroplasts in the Potato: WINONA E. STONE. Illus.	421

	Page
Some Nemic Parasites and Associates of the Mountain Pine Beetle (<i>Dendroctonus monticolae</i>): G. STEINER. Illus.....	437
The Vitamin A, B, C, and G Content of Concord Grapes: ESTHER PETERSON DANIEL and HAZEL E. MUNSELL. Illus.....	445
Effect of Carbon Dioxide Content of Storage Atmosphere on Carbohydrate Transformation in Certain Fruits and Vegetables: ERSTON V. MILLER and CHARLES BROOKS. Illus.....	449
Rhizoctonia Bottom Rot and Head Rot of Cabbage: F. L. WELLMAN. Illus.....	461
The Digestive Enzymes of the Colorado Potato Beetle and the Influence of Arsenicals on Their Activity: DAVID E. FINK.....	471
A Study of Several Factors in the Separation of Serum from Bottled Cream: G. MALCOLM TROUT and J. C. McCAN. Illus.....	483
Inheritance of Resistance to Bunt, <i>Tilletia tritici</i> , in Hybrids of White Federation and Odessa Wheat: FRED N. BRIGGS. Illus.....	501
The Effect of Artificial Drying on the Availability of the Nutrients of Alfalfa Hay: E. B. HART, O. L. KLINE, and G. C. HUMPHREY.....	507
A Cytological Study of Heterothallism in <i>Puccinia coronata</i> : RUTH F. ALLEN. Illus.....	513
Some Physiological Studies of Potatoes in Storage: R. C. WRIGHT. Illus.....	543
Apparent Digestibility of, and Nitrogen, Calcium, and Phosphorus Balance of Dairy Heifers on, Artificially Dried Pasture Herbage: R. E. HODGSON and J. C. KNOTT.....	557
An Empirical Test of the Approximate Method of Calculating Coefficients of Inbreeding and Relationship from Livestock Pedigrees: JAY L. LUSH. Illus.....	565
Oxidation-Reduction Potentials and the Hydrogen-Ion Concentration of a Soil: L. G. WILLIS. Illus.....	571
Quality, Size, Capacity, Gross Anatomy, and Histology of Cow Udders in Relation to Milk Production: W. W. SWETT, FRED W. MILLER, R. R. GRAVES, and G. T. CREECH. Illus.....	577
Relation of the Semipermeable Membranes of the Wheat Kernel to Infection by <i>Gibberella saubinetii</i> : GRACE WINELAND PUGH, HELEN JOHANN, and JAMES G. DICKSON. Illus.....	609
A Three-Year Study of the Chemical Composition of Grass from Plots Fertilized and Grazed Intensively: J. G. ARCHIBALD, P. R. NELSON, and E. BENNETT. Illus.....	627
Comparison of Conformation, Anatomy, and Skeletal Structure of the Cow and Bull of a Dairy Breed: W. W. SWETT, R. R. GRAVES, and FRED W. MILLER. Illus.....	641
Efficiency Factors and Their Use in Determining Optimum Fertilizer Ratios: W. A. HUELSEN. Illus.....	675
Efficacy of Different Strains of <i>Brucella abortus</i> as Immunizing Agents against Infectious Abortion: W. E. COTTON.....	705
Distribution of the Cotton Root-Rot Fungus in Soil and in Plant Tissues in Relation to Control by Disinfectants: C. J. KING and CLAUDE HOPE. Illus.....	725
Determination of the Errors of Estimate of a Forest Survey, with Special Reference to the Bottom-Land Hardwood Forest Region: FRANCIS N. SCHUMACHER and HENRY BULL. Illus.....	741
Definitions of Honey Color Grades: E. F. PHILLIPS.....	757

ERRATA AND AUTHORS' EMENDATIONS

Page 8, fifth line from bottom, "Red Indian" should be "Hidatsa Red."

Page 48, Table 5, third column, line 3, "42" should be "47"; line 4, "46" should be "45."

Page 190, fifth line from bottom, "502" should be "500"; and second line from bottom, "500" should be "502."

Page 272, Table 4, line 1, under No. 13, "+2=2" should be "+2+2."

Page 282, sixteenth line from bottom, "Seventy-three" should be "Seventy-five."

Page 323, line 17, "Figure 1, B" should be "Figure 1, C."

Page 328, twenty-second and twenty-third lines from bottom, "(pl. 2, A)" should be "(fig. 1, C)."

Opposite page 332, legend for Plate 6, line 7, "(fig. 3, B)" should be "(fig. 2, B)"; line 8, "Figure 3, A" should be "Figure 2, A."

Page 333, line 21, "(p. 65)" should be "(p. 325)."

Page 426, Plate 5 should be turned 180 degrees.

Page 443, the female formula should be:
$$\text{Female. } \frac{0.5 \quad 3.5 \quad 4.17 \times 82.16 \cdot 99.0}{0.4 \quad 0.8 \quad 0.8 \quad 1.2 \quad 0.9} \cdot 2.4 \text{ mm.}$$

Page 456, Table 3, column 2, under CO₂, "45-47" should be "35-47"; last line of text, "starch" should be "polysaccharides."

Page 618, Figure 5, B, "st" should have been inserted in the upper left-hand corner.

Page 619, legend for Figure 6, D, last line, "350" should be "467."

Page 628, seventh line from bottom, "form" should be "farm."

Page 682, Table 2, mean phosphorus efficiency factor for the series 020 021 022-024 in sixth column under (Green sweet corn, "18.10±2.0," should be "18.10±2.2."

Page 688, Table 4, potash efficiency factor for treatment 112, "27.63±6.3," should be "27.63±6.4."

Page 691, Table 5, nitrogen efficiency factor for treatment 122 under Edible green sweet corn, "25.95±9.8," should be "25.95±9.3."

Page 696, Table 7, under Green sweet-corn fodder, the mean increase in yield for the series 012-112-212-412, "23.79±27," should be "23.79±2.7."

Page 702, second line of last paragraph, formula in parenthesis "(7.5-5-64-50)" should be "(7.5-64-50)."

Page 706, second line from bottom, insert "local" between "the" and "reactions."

Pages 714 and 715, footnotes to Tables 5 and 6, reference marks "c" and "c" should be reversed.

Page 721, heading for Table 9, "a virulent" should be "an avirulent."

Page 724, twenty-first line from bottom, insert "to" between "abortion" and "bovine."

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 45

WASHINGTON, D. C., JULY 1, 1932

No. 1

A CLASSIFICATION OF THE VARIETIES OF FIELD BEANS, *PHASEOLUS VULGARIS*¹

By F. H. STEINMETZ, formerly Assistant Agronomist, and A. C. ARNY, Associate Agronomist, Minnesota Agricultural Experiment Station

INTRODUCTION

Several classifications of both European and American varieties of field beans are available, but up to the present none deal specifically with species of *Phaseolus* and varieties of *P. vulgaris* L. grown for their dry edible seeds. The field-bean-growing sections are somewhat localized in the New England, the North Central, and the Pacific Coast States ranging from Oregon to southern California and Arizona. Since the range in climate between any two extremes of the above-mentioned sections is wide, it follows that varieties grown in one extreme are not always adapted to another. The choice of a variety adapted to local conditions affects directly the yield and profitability of the crop; hence the choice of a variety for a given locality merits careful consideration. However, in order to be able to make this choice, the identity of types and varieties must be established with certainty. There is need, therefore, for a classification of field varieties by the use of which the identity of the varieties may be definitely established. It is the purpose of this study (1) to determine a practical basis for the classification of field varieties of common beans, (2) to construct a key based largely upon characters that are stable under varying climatic conditions so that it may be of value wherever beans are grown, and (3) to describe the varieties as found when grown in the environment of University Farm, St. Paul, Minn.

REVIEW OF LITERATURE

Von Martens (10)² published his second revised classification of garden beans in 1869. He did not distinguish garden beans from field beans. In his classification the common beans (*Phaseolus vulgaris*) are separated into seven species according to the shape of the seed. A secondary division is based upon the color of the seeds.

In 1865 Burr (3, p. 434-481) described 56 varieties of *Phaseolus vulgaris*. Eight of these appear to be field varieties recognized at present.

Irish (7) described all obtainable varieties from cultures grown at the Missouri Botanical Garden in 1901. His key to varieties is based primarily on the form and color of the seed. For secondary divisions he uses plant and immature pod characters.

The use of dry seeds and green pods is impractical for the reason that the immature pods can not be stored conveniently for future use in identification. The reliability of absolute measurements may, at least, be questioned. However, the combined use of seed, pod, and type of plant is a step toward what undoubtedly will become a

¹ Received for publication Dec. 28, 1931; issued August, 1932. Paper No. 1070 of the Journal Series of the Minnesota Agricultural Experiment Station.

² Reference is made by number (italic) to Literature Cited, p. 49.

comprehensive basis for the identification of varieties. Irish states that the variety Navy is largely grown as a field crop.

In 1907 Tracy (13) described American varieties of garden beans, *Phaseolus*, within three species—*vulgaris*, *lunatus*, and *multiflorus*. His major divisions are based upon plant habit and character of green pod. A secondary division is based upon form, size, and color of the dry seed. He includes varieties of field beans. In his key the varieties of field beans are set apart according to texture of the green pod.

Tracy has made a fairly clear distinction between field and garden varieties of common beans based upon the texture of the green pods. However, when this comparison is extended to the dry mature pod the distinction becomes more apparent. Dry pods of field beans are fibrous and dehisce at the sutures, while the dry pods of garden varieties of garden beans are brittle, contain relatively little fiber, and break in cross section rather than dehisce at the sutures during threshing. The terms "thick stemmed" and "thin stemmed" are too indefinite to be of value in placing plants with stems intermediate in thickness. Tracy refers specifically to 12 varieties as field beans in his descriptions.

Jarvis (8) described American varieties of beans, which he separated into three species of the genus *Phaseolus* and four other genera—*Vicia*, *Vigna*, *Dolichos*, and *Glycine*. He utilizes color and form of seed, but bases his major classification upon size of seed and the ratio of the length to the width. In his descriptions he groups varieties of *Phaseolus* according to the type of the plant and the pods.

In the exclusive use of seed characters for the classifications of varieties Jarvis makes many distinctions so minute as greatly to lessen the practical usefulness of his key. The combined use of seed, dry pod, and plant habit in a key would appear to facilitate greatly the identification of varieties. In his key he makes no separation of garden varieties and field varieties. However, in his descriptions he points out whether a variety is suited for green-pod or for dry-shell purposes. He refers specifically to 21 varieties as field types.

In 1912 Freeman (5) described six native field varieties of *Phaseolus vulgaris*, namely, Pink, Bayou, Hansen, Garaypata, Red Indian, and Mottled Red Indian. In addition, he first describes the tepary bean, *P. acutifolius* var. *latifolius* as a new field species. There is incorporated a detailed discussion of the origin of this new species. He concludes that it is a native of the southwestern part of the United States or Mexico. He adopts the name "tepary," which the Mexicans use to distinguish it from the common "frijole" or kidney bean. Three varieties of "teparies" are described, but he states that as many as 49 distinct forms are recognized.

In 1912 Hendry (6) described 16 varieties of field beans adapted to California, representing four genera—*Vigna*, *Vicia*, *Cicer*, and *Phaseolus*. He gives the history and the adaptation of the varieties described.

In 1919 Chittenden (1) grew 253 stocks of beans of which 224 are described as dwarf or nonclimbing types. The remaining 29 climbers or semiclimbers are not described.

The method used is undoubtedly valuable as a convenience to growers and seed producers but is limited in usefulness because flowers and green pods are available only during a part of the growing season.

MATERIALS AND METHODS

As early as 1914 a collection of seed of varieties of field beans was begun. Seed from the bean-growing sections of the United States and Canada was obtained through seed houses, growers, and experiment stations. An effort was made to obtain seed of the main varieties grown in each of the bean-growing sections of the United States, namely, the New England, north central, Pacific coast, and south-western sections. In addition, a few samples were obtained from Sweden and France. The growing of these varieties began in 1915. Intensive work toward isolating types began with the harvesting of the 1918 crop.

From the beginning the beans were planted in rows 18 feet long and 2 feet apart with one seed dropped every 3 inches. In order to fix the type for a given variety, commercial seed was planted in rows as described above. At harvest time typical and nontypical plants were selected and the seed planted in progeny rows for observation and increase. The rows having plants which were the most representative of a certain variety were chosen as the type. Other rows representing other types in the original sample were continued if they were sufficiently distinct. From the type rows the one showing the best performance record was increased, given a Minnesota number, classified, and described.

It is recognized that some natural crossing occurs in beans when varieties are grown near each other. The only effort made to control the effects of this was to rogue out plants which varied from the selected type.

During three years of the study, when it was possible, 100 pods were selected from the various types at harvest time and stored in small pasteboard boxes. These pods were measured, the length and width of pod and the length of spur being taken. From the same material the number of seeds per pod was determined and the three dimensions of 100 seeds were obtained. The weight per 1,000 seeds was determined from the bulk of the seed which was harvested from each row or plot. The seeds were counted into two lots of 500 each for weighing.

Weights and measurements of the seeds of varieties are given in Tables 1-4, inclusive.

EFFECT OF ENVIRONMENT UPON SIZE OF SEED

Among the factors affecting the size of the seed are temperature, moisture, productivity of the soil, rate, and date of planting.

Freeman (5), Jarvis (8), and Hendry (6) use size of seed in their descriptions of varieties and they report the measurements of three dimensions. Zavitz (14) and Freeman (5) give weight of seed as a descriptive character. A summary of the measurements and weight of seed reported for a few varieties of beans grown in different localities is shown in Table 1. By comparing the measurements recorded it will be seen that marked variations occur. For example, the length of seeds in different years for the variety Red Kidney ranges from 1.8 cm in New York to 1.4 cm in California. The weight per 1,000 seeds for different years as reported by Zavitz and by the present writers for the variety Red Kidney shows a wide variation in size. A study of this table indicates that a classification based mainly upon size of seed may well be questioned, except possibly for a particular locality.

It was arranged to have some of the bean varieties grown at outlying stations in Minnesota; that is, at Crookston, Grand Rapids, Duluth, Coon Creek, and Radisson. By the use of the seed produced at these locations a study was made of the effect of environment upon size. From the results given in Table 2 it is evident that for two varieties grown in 1920 there is a wide variation in the size of the seed. Robust beans were approximately 40 per cent smaller by weight grown on peat at Radisson³ than at Grand Rapids on a sandy loam. The difference is not so marked between any other two localities, but it is great enough to make it questionable whether a useful classification of Robust beans for different localities or environments can be based upon the size of the seed. The seed of the variety Improved Goddard grown at Crookston is approximately 30 per cent by weight less than seed grown at Grand Rapids. The above evidence indicates that the size of the seed produced from the same varieties is materially affected by the locality in which it is grown.

At University Farm, St. Paul, Minn., some work was done upon rate and date of planting. The results are given in Table 3. The seeds produced in 1920 from Robust 76 beans planted late are smaller in size than those planted at the regular time, and those spaced far apart in rows are larger than those planted close. For example, Robust beans planted May 12 weighed 233 g per 1,000 and those planted May 22 weighed 212 g per 1,000, while the late planting, June 22, weighed 204 g per 1,000. It appears, therefore, that date of planting influences the size of seed produced. The other factor, rate of planting, gave similar results. Robust beans planted 4 inches apart in rows produced seed weighing 218 g per 1,000, while beans planted 8 inches apart produced seed weighing 244 g per 1,000.

TABLE 2.—Variation in size of seed of beans of two varieties grown in different localities in 1920

Variety	Locality	Length of seed	Width of seed	Thick- ness of seed	Ratio			Thick- ness, width factor	Weight per 1,000 seeds
					Length	Width	Thick- ness		
		<i>Centi- meters</i>	<i>Centi- meters</i>	<i>Centi- meters</i>					<i>Grams</i>
Robust No. 76...	University Farm...	0.840	0.584	0.480	1	0.195	0.571	0.822	170.8
	Duluth.....	.849	.625	.508	1	.736	.598	.813	185.8
	Crookston.....	.803	.651	.539	1	.739	.603	.828	205.2
	Grand Rapids....	.872	.682	.564	1	.782	.647	.827	236.2
	Coon Creek, sand..	.840	.577	.452	1	.687	.539	.783	157.4
	Radisson, peat...	.785	.569	.446	1	.725	.568	.787	133.0
Improved God- dard No. 139.	Duluth.....	1.603	.798	.593	1	.498	.369	.743	529.0
	Crookston.....	1.522	.727	.565	1	.477	.371	.777	380.8
	Grand Rapids....	1.615	.818	.694	1	.507	.374	.738	550.0
	University F.rm...	1.529	.765	.534	1	.500	.349	.698	435.7

³ Private farm near St. Paul, Minn.

TABLE 3.—*Variation in size of seed of Robust No. 76 beans grown at University Farm when planted at different rates and dates in 1920*

Date of planting	Rate of planting	Length of seed	Width of seed	Thick-ness of seed	Ratio			Thick-ness, width factor	Weight per 1,000 seeds
					Length	Width	Thick-ness		
		Centi-meters	Centi-meters	Centi-meters					Grams
May 5	1 seed each 4 inches	0.935	0.662	0.551	1	0.708	0.589	0.832	217.7
May 5	1 seed each 8 inches	.949	.673	.569	1	.709	.589	.831	243.8
May 12	1 seed each 3 inches	.945	.677	.562	1	.716	.594	.830	233.0
May 22	do	.924	.645	.527	1	.698	.570	.817	211.6
June 22	do	.901	.651	.597	1	.723	.597	.826	204.4

The growing season of 1919 was favorable throughout at University Farm, but the season of 1920 was less so. Until about July 10 there was heavy precipitation, but during the remainder of the growing season rainfall was scant. The crop of beans produced was affected differently during its development in the two seasons mentioned, as seen in Table 4. Robust beans produced in 1919 weighed 227 g per 1,000, while seeds of the same variety produced in 1920 weighed 177 g per 1,000. The variety Improved Goddard does not show similar variation. Seeds produced by this variety in 1919 weighed 434 g per 1,000, while seeds produced in 1920 weighed 436 g per 1,000. The variety Improved Goddard is an early type, while the variety Robust matures approximately two weeks later when planted at the normal date. At University Farm, Robust beans matured uniformly when planted as late as July 1, while Snowflake beans were nonuniform in maturity. It is probable that Improved Goddard matured before the drought had progressed far enough to hinder normal development, whereas Robust was hampered by the drought. The Robust beans grown on a very sandy soil at Coon Creek in 1920 weighed 157 g per 1,000. These were checked in development by the drought. From the evidence given it appears that seasonal variation may be great enough to make it impossible to base a classification of beans upon size of seed with the expectation that it will be of use over a period of years or in widely different locations.

CHARACTERS USEFUL IN CLASSIFICATION AND DESCRIPTION

The observations here reported are based upon three years' work at University Farm.

SEEDLING CHARACTERS

The term "seedling leaves" as here used refers to the first pair of leaves above the cotyledons. The size of seedling leaves produced is closely correlated with the size of the seed.

TABLE 4.—*Variation in size of two varieties of beans grown at University Farm in 1919 and 1920*

Variety	Year	Length of seed	Width of seed	Thick-ness of seed	Ratio			Thick-ness, width factor	Weight per 1,000 seeds
					Length	Width	Thick-ness		
		Centi-meters	Centi-meter	Centi-meter					Grams
Improved Goddard, No. 139.	1919	1.580	0.797	0.539	1	0.504	0.341	0.710	433.8
	1920	1.529	.765	.534	1	.500	.349	.698	435.6
Robust No. 76.	1919	.912	.669	.567	1	.733	.622	.847	227.1
	1920	.840	.584	.480	1	.695	.571	.822	176.8

Color of seedling leaves and stem is correlated with color of seed coat. In the brown-seeded varieties the stem is tinted brown, especially close to the surface of the soil. The same color distribution is true of red-seeded varieties. In the solid black or black mottled varieties under observation a purple tint extends into the veins of the seedling leaves. In all except the solid black or black mottled varieties the color soon disappears. However, in the latter group the purple color remains until maturity, especially in the stems. There are varying shades of green, but this occurs in both white-seeded and colored-seeded varieties. The tepary and Lima beans have distinctly dark green seedling leaves. The usefulness of this character is merely supplementary in description.

Freeman (5) has pointed out that the length of seedling-leaf petiole is an easy method of distinguishing tepary beans from common beans. The petiole is very short in the former and considerably longer in the latter.

The character of the seedling-leaf surface is useful in identifying a few varieties of the common field beans. The seedling-leaf surface in a majority of the varieties is smooth. However, there are marked gradations of smoothness, ranging from smooth in Navy Pea to very wrinkled in Vineless Marrow. The latter variety is readily distinguished by this character from White Marrow, a long trailing variety having seeds similar in size and appearance.

Pubescence of the seedling leaf surface is useful largely in distinguishing species of the genus *Phaseolus*. Varieties of the species *acutifolius* and *lunatus* have glabrous leaf surfaces while varieties of *vulgaris* and *multiflorus* have pubescent leaf surfaces.

Seedling characters are of limited value in the identification of varieties.

CHARACTERS OF THE WELL-DEVELOPED PLANTS

HABIT OF GROWTH

In habit of growth bean plants may be classified as bush (fig. 1) and trailing (fig. 2). The latter range from short to long trailing, while the former have less range in height due to their habit of growth.

Bush beans are characterized by a determinate growth habit. The plants are erect and have from five to eight nodes in the main axis and terminate in an inflorescence. Emerson (4), p [4] gives the characteristics of bush beans as follows:

The main axis is terminated by an inflorescence when from about four to eight internodes have developed and can not be forced to make further growth tho provided with the most favorable conditions of moisture and temperature, and even tho the flowers be removed to prevent the drain of seed production.

He believes that the common bush bean is a mutation from the pole bean.

Trailing beans have an indeterminate habit of growth. The plants range from erect (fig. 3) to decumbent (fig. 4) in habit. They may also be grouped as short and long trailing. The short-trailing forms have from 11 to 16 nodes, while the long-trailing forms may have as many as 28 or 30. The first flower clusters appear rather near the base of the stem and the others progressively higher as new nodes are formed. Emerson (4) found that if favorable conditions for growth are provided and if heavy seed production is prevented, pole beans can be kept growing for a long time.

HEIGHT

Height obviously is determined by number of nodes and internode length. Some trailing varieties have long internodes, others have short ones. For example, White Marrow, in 1920, had an average



FIGURE 1.—Bush habit of Improved Goddard

of 17 nodes with an internode length of 7.8 cm, while Red Indian had the same number of nodes with an average internode length of 4 cm. There appears to be a similar variation in bush varieties. For example, in 1920, Red Kidney had an average of 7 nodes with an internode length of 6.4 cm, while Selection No. 90 had an average of

6 nodes with an internode length of 5.7 cm. Emerson (4) reports the existence of distinct types of bush beans with respect to both number and length of internodes. He found this to hold for pole



FIGURE 2.—Trailing habit of the Navy Pea, variety Robust

beans. Data reported in this study were taken when the plants had reached approximately their maximum height. The measurements were taken of representative plants and recorded in centimeters. The number of nodes was determined by averaging the actual count

of 10 representative plants. Both of these determinations were made for the years 1919 and 1920.

COLOR OF LEAVES AND STEMS

The color of fully developed leaves and stems in the majority of the varieties is green throughout. However, in some dark-seeded varie-

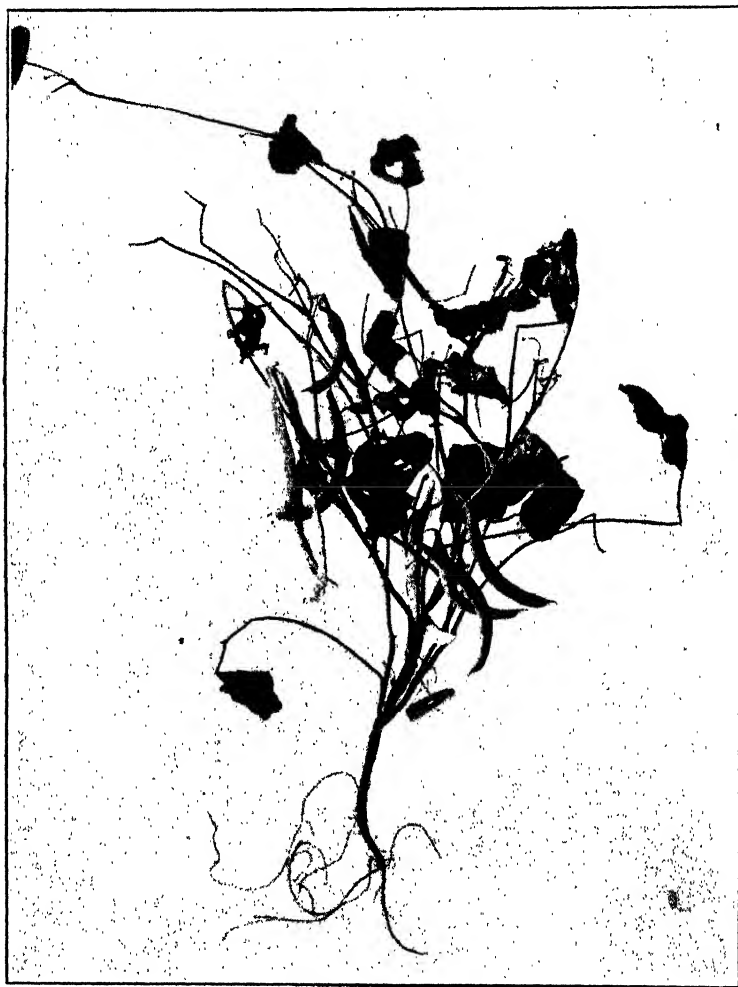


FIGURE 3.—Erect trailing habit of Black Turtle Soup

ties, such as Black Turtle Soup, Zebra, and Selection No. 121, the stems are tinted purple. These same varieties have dark-green leaves. On the other hand, Lady Washington, a white-seeded variety has dark-green leaves. Consequently there appears to be no correla-



FIGURE 4.—Decumbent habit of Great Northern

tion between the intensity of green color of the leaf and the color of the seed.

SIZE AND SHAPE OF LEAVES

The size of the mature leaf varies closely with the size of the seed. On a single plant, leaves vary from small near the growing tip to large where they are fully developed. Previous writers have used size of the leaf in the description of varieties. Irish (7) gives the size

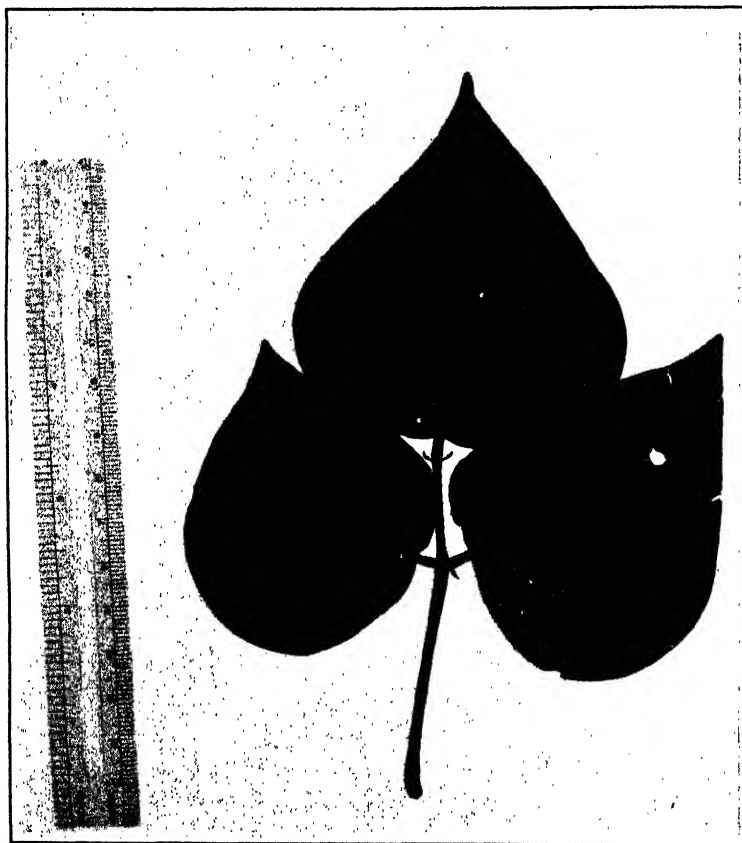


FIGURE 5.—Fully developed leaf of the Navy Pea, variety Robust

in actual measurements while Tracy (13) and Jarvis (8) use such general terms as large, medium, and small. Freeman (5) gives averages of 100 mature leaves taken at random for length and breadth to show that tepary leaves are smaller than common bean leaves. Hendry (6) tabulates length and breadth dimensions for the median leaflet of the varieties grown in California. The use of absolute measurements in the description of varieties with a species appears to be impractical because of the wide variation in size of the leaf on a single plant. In these descriptions all determinations of size were

made on fully developed leaves of the variety concerned. The leaf shown in Figure 5 is described as small, that shown in Figure 6 as medium, and that shown in Figure 7 as large.

Mature leaves vary in shape and character of the surface. The leaf shown in Figure 5 is described as small and short pointed, that shown in Figure 6 as medium and long pointed, and that shown in Figure 8

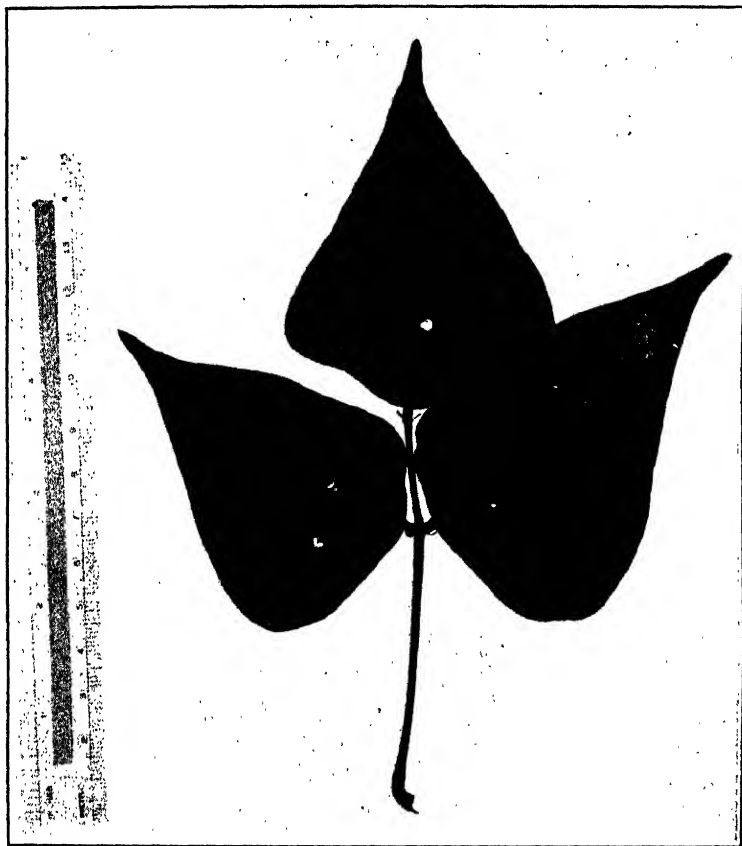


FIGURE 6.—Fully developed leaf of the variety Ruby Horticultural Bush

as broad and short pointed. Mature leaf surfaces are referred to as crumpled (fig. 9) and as smooth (fig. 8).

COLOR OF FLOWERS

Color of blossom was determined by careful comparison of newly opened flowers with the colors of a standard chart (12). Since there is a variation in color in the different parts of the corolla, the color was determined for the standard and the wings. Previous writers have recognized color of blossom, but have usually described it in general terms such as "pink" or "violet." Tracy (13) refers to the

use of a color chart published by Henri Dauthenay, but does not give specific citation to color plates.

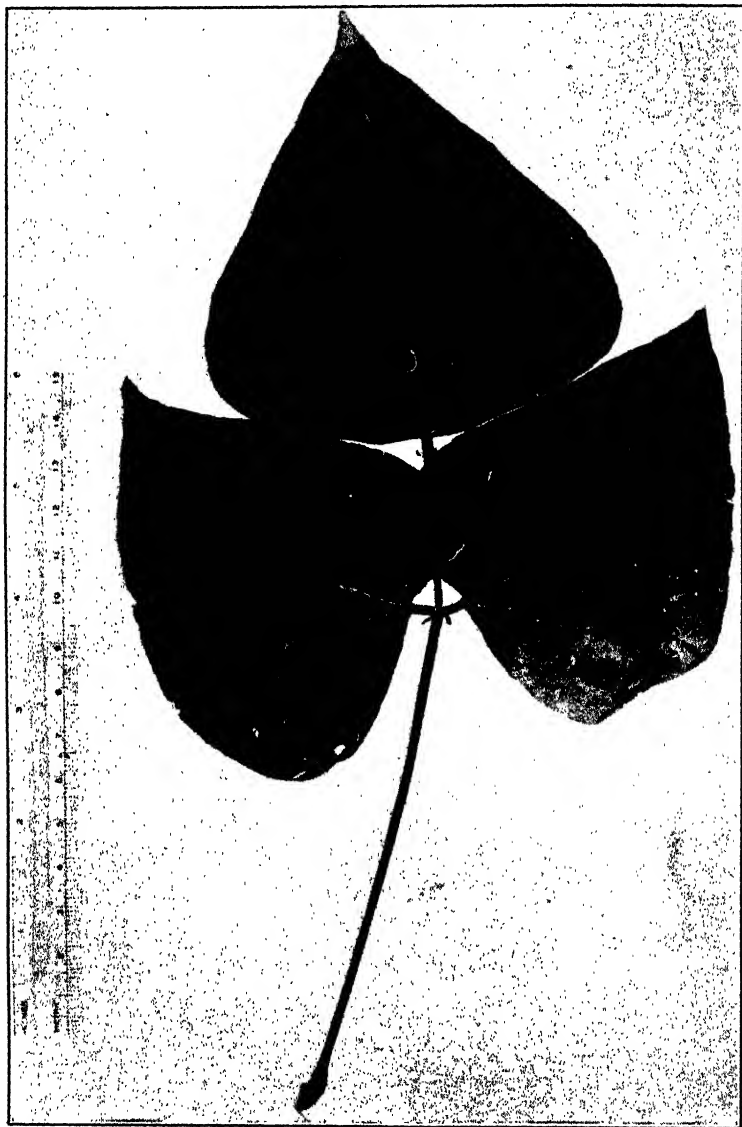


FIGURE 7.—Fully developed leaf of the variety White Marrow

TIME OF MATURITY

Time of maturity is of considerable economic importance, for the length of the growing season limits the growing of some types of

beans which are late in maturing. In identifying varieties otherwise similar in seed and plant characters, the time of maturity is of decided importance. For example, Snowflake and Robust have seed and plant characters which are similar, but the former matures approximately two weeks earlier than the latter when planted at the normal date. Time of maturity as referred to in this study applies to environmental conditions as they are at University Farm.

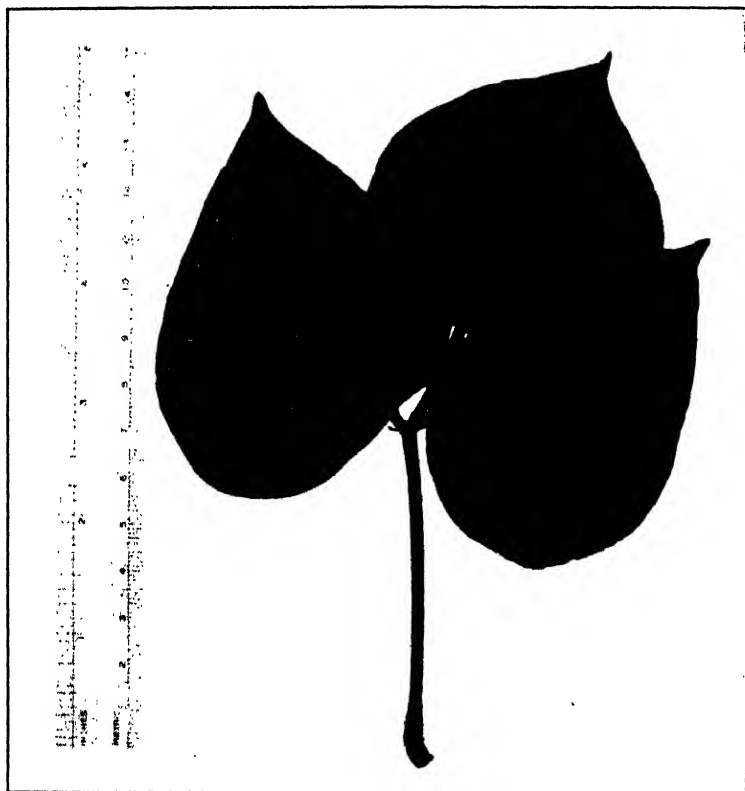


FIGURE 8.—Fully developed leaf of the variety Arikara Yellow

PRODUCTIVENESS

As a descriptive character productiveness undoubtedly merits consideration. Jarvis (8) and Tracy (13) use comparative terms to express yields of green snap pods. Hendry (6) and Freeman (5) give yields in tabular form showing the relative yielding power of the varieties described in their localities. Data on yielding power are of necessity restricted to a given locality. In this discussion four degrees of comparison are used, namely, low, medium, productive, and very productive. In the majority of cases these comparisons are based upon plot tests (8 by 18 feet) repeated four times over a period of three to four years.

DISEASE RESISTANCE

Disease-resistance data are reported for the crop years 1919, 1920, and 1922. Anthracnose (fig. 10) was the most serious disease prevalent. Leach (9) found at least eight distinct biologic forms of this disease. The data are based upon injury to the green pods. It is apparent from the figures shown that there is a wide variation in the susceptibility of varieties to anthracnose. Blight (fig. 11) was less prevalent, and notes were taken on its development only on the

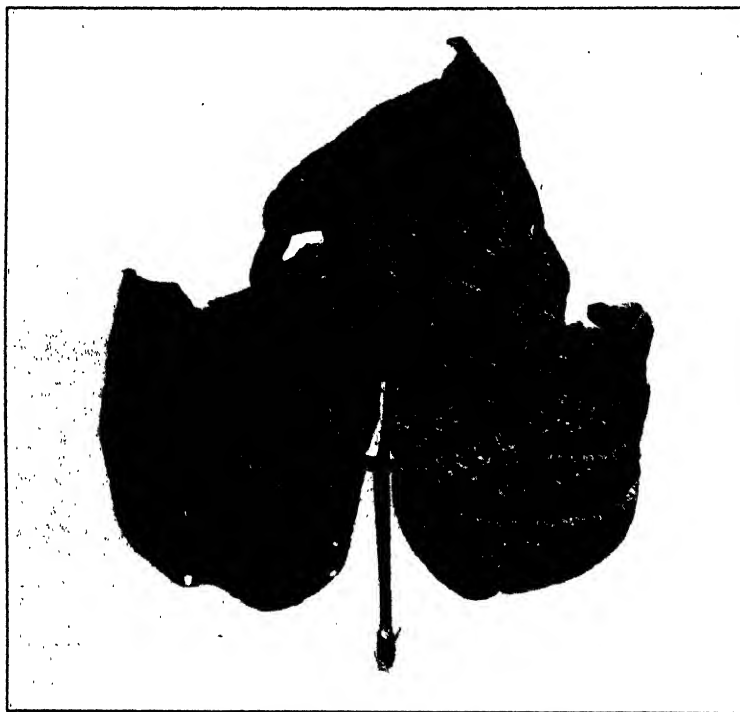


FIGURE 9.—Fully developed leaf of the variety Eureka

green pods. Leaf rust occurred on several of the late-maturing varieties in 1919 and 1921. (Fig. 12.) Under the rather humid condition which prevailed throughout the growing season of 1919 anthracnose did considerable damage, but the rather dry growing season of 1920 apparently checked its development.

POD CHARACTERS

Characters of the pods are not influenced so much as the vegetative parts by the environment and therefore are more useful in distinguishing varieties.

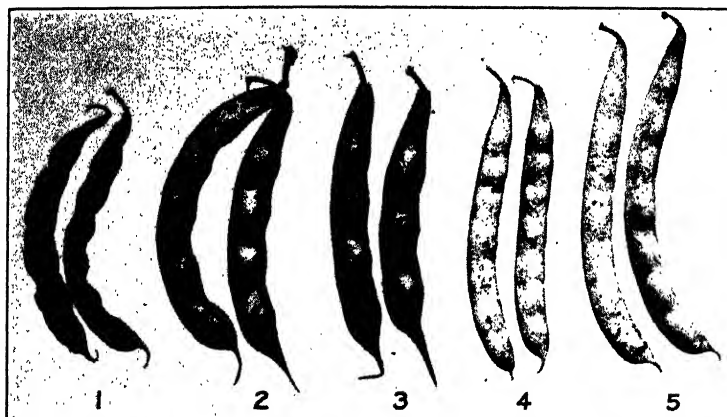


FIGURE 10.—Anthracose on dry pods of Hidatsa Red (1), Vineless Marrow (2), Ruby Horticultural Bush (3), Robust (4), and Black Turtle Soup (5)



FIGURE 11.—Pods of Red Kidney showing blight injury



FIGURE 12.—Leaf-rust pustules on upper and lower leaf surfaces of beans



FIGURE 13.—Colored mature pod of Selection No. 148 (1) and colorless mature pod of Arikara Yellow (2)

COLOR

Many varieties develop a characteristic pod color just as they approach maturity. Selection No. 148, for example, shows very marked purple-splashed areas (fig. 13) which are permanent, while Red Kidney (fig. 14-1), which often has red-tinged areas on the immature pods usually shows no color in the dry mature state. Great Northern, a white-seeded variety, often develops purple-splashed areas which are permanent. Color in pods is described as found at the period approaching maturity. Observations in these cultures indicated no consistent correlation between pod color and other coloration in the plant. This is in accord with the findings of Shaw and Norton (11).

SHAPE

Shape of pods has been used in descriptions by Irish (7) and Tracy (13). The former introduces into his classification shape of pods as seen in lateral view, while the latter introduces the shape of pod as seen in cross section. Both of these workers considered the pod as found in the immature condition. Since pod characters are rather fixed, both lateral and ventral views are used in this classification. In the lateral view pods are described as long and straight (fig. 15-1), short and straight (fig. 15-3), long and curved (fig. 15-2), short and curved (fig. 15-4), and as curved at the free end and reflexed at the stem end (fig. 15-5). In ventral view the pods are referred to as flat (fig. 16-2) and as rounded (fig. 16-1). Pods which are broad in ventral view are described as rounded.

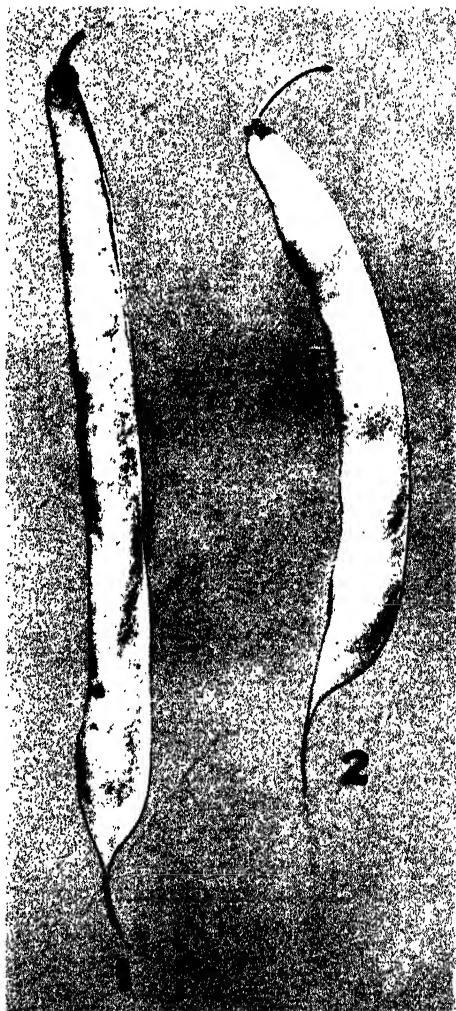


FIGURE 14.—Colorless mature pods of Red Kidney (1) and Selection 90 (2), showing tapering pod apices

TEXTURE

Mature pods of field beans vary in texture from coarse, thick, and fibrous to thin, papery, and fibrous. The coarse type is represented by Vineless Marrow (fig. 17-1) and the papery type by Great Northern (fig. 17-2).

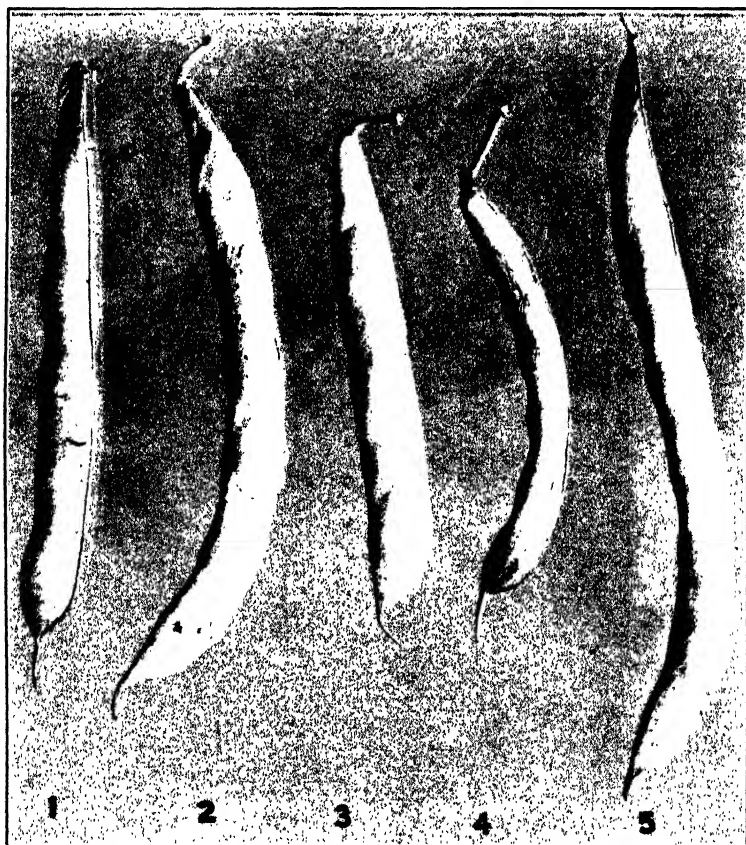


FIGURE 15.—1, Long, straight pod of Red Kidney; 2, long curved pod of Haricot; 3, short straight pod of Selection 108; 4, short curved pod of Brown Swedish 144; 5, pod curved at free end and reflexed at stem end of Selection 162

LENGTH AND WIDTH

Length and width of pod have been used in descriptions by previous writers. The general terms "long" and "short" are employed. Tracy (13) gives approximate length in inches of snap pods. Hendry (6) tabulates pod dimensions in length, breadth, and thickness. In the present descriptions it seemed desirable to present actual measurements for length and width of the dry mature pods made with a caliper to tenths of a centimeter. Length was measured from the tip of the spur to the juncture of the pod and the pedicel.

Width was measured at the widest axis, which was found to be at approximately the second seed from the spur. Pods measuring 0.9 cm or less in width are considered narrow and all over 1.1 cm as wide. Pods with widths in between these arbitrary limits are considered intermediate.

APEX

Pod apex as used in these descriptions is that portion of the pod between the base of the spur and the point where the pod begins to taper toward the free end. Apparently previous writers have not distinguished between pod apex and spur. For example, in describing the pod of White Kidney, Tracy (13) uses the expression "point of pod medium in length and straight," while Jarvis (8) uses the expression "moderately stout straightpoint." From these quotations it seems that it can be safely inferred that they had reference to the pod spur. Differences in pod apex may readily be seen by examining the apex of Selection No. 90 (fig. 14-2) which tapers off gradually toward the spur, while in Selection 109 the apex tapers off rather abruptly toward the spur (fig. 15-3). The former type of apex is described as elongated and the latter as abrupt.

SPUR

The term "spur" as used in these descriptions refers to the narrow projection at the free end of the pod beginning where the pod ceases to be hollow. This point was determined in order to have a somewhat definite point from which to measure the length of the spur. When the spur extends in line with the ventral suture, it is described as marginal. (Fig. 15-3.) When it extends from a point not in line with the ventral suture, it is described as not marginal. (Fig.



FIGURE 16.—Rounded pod of Vineless Marrow (1) and flat pod of Pearce Improved (2)

15-1.) Spurs are curved (fig. 15-3) or straight (fig. 15-1). Straight spurs are those which extend approximately in a straight line with the longitudinal axis of the free end of the pod. Curved spurs are those which bend away from a straight line with the longitudinal axis of the free end of the pod toward the dorsal suture.



FIGURE 17.—Coarse pod of Vineless Marrow (1) and thin, papery pod of Great Northern (2)

DRY-SEED CHARACTERS

Dry seeds are used exclusively in Jarvis's (8) key for the classification of American varieties of beans. All writers have given seeds a prominent place in description, which is in accord with the practice reported in this study.

SIZE

Size of the dry seeds as reported in this discussion is expressed in their three dimensions and number of seeds per 1,000 g. Each measurement was taken with a micrometer at the broadest axis and read to 0.01 mm. The measurements reported in this study are largely based on 100 determinations. In the majority of cases the weighings represent the actual weight of 1,000 seeds. All weighings were made on a No. 17 Troemner balance.

SHAPE

Shape of dry seeds has been recognized by the majority of writers as a characteristic of value in classification. Von Martens (10) based his major groups upon shape, while Jarvis (8) recognized work done by Gilmore at Cornell in grouping field beans into four market groups, namely, kidney, marrow, medium, and pea. In the present classification seeds are grouped as reniform (fig. 18-1, 2) and not reniform (fig. 18-3 to 6, inclusive). In cross section the seeds are described as flat (fig. 19-1, 2), intermediate (fig. 19-3, 4), and round (fig. 19-5, 6). From the measurements taken a single factor was

derived by dividing the thickness by the width to designate whether the seeds were flat, intermediate, or round. Those with a thickness factor less than seven-tenths are classified as flat, those falling between seven-tenths and eight-tenths are grouped as intermediate, and those with a factor of eight-tenths or over are designated as round. In the descriptions of a few varieties seeds are described as oval (fig. 18-3) and as spherical (fig. 18-4). The ends of seeds are described as round (fig. 18-2, 4) and as truncated (fig. 18-5 and 6).

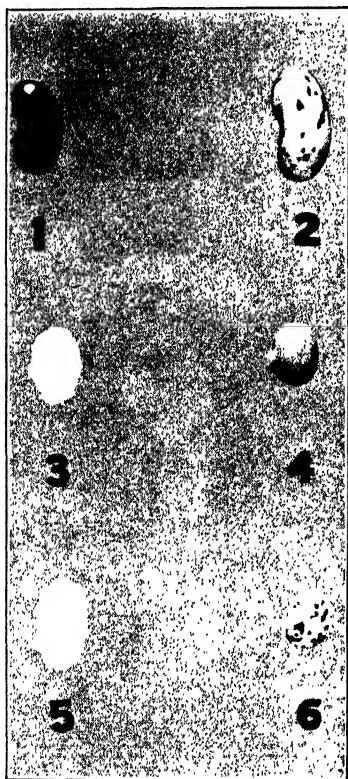


FIGURE 18.—Bean seed of the following varieties: 1, Black Turtle Soup; 2, Improved Goddard; 3, Vineless Marrow; 4, Eureka; 5, Pearce Improved; 6, Wild Goose

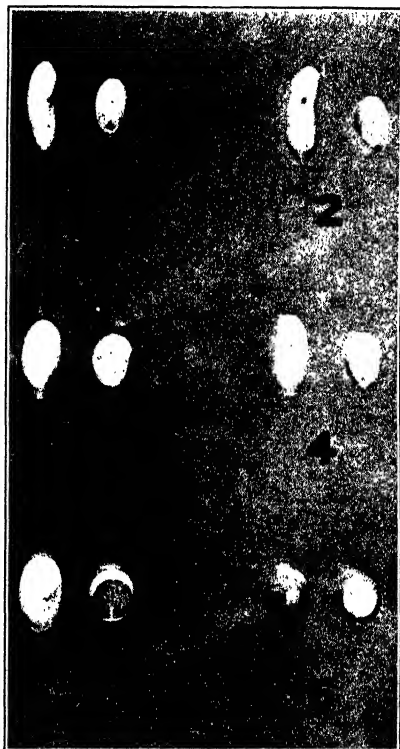


FIGURE 19.—Bean seed and cross sections of the following varieties: 1, Haricot; 2, Great Northern; 3, Burlingame; 4, Selection No. 106; 5, Vineless Marrow; 6, Eureka

COLOR

Color of dry seeds has been used to distinguish varieties by Von Martens and others. Chittenden (1) at Wisley places seed color second to pod texture in classifying Dwarf French beans. All writers have recognized color in a general way, but Freeman (5) was the first to give specific citations to a standard color chart. In these descriptions beans are arranged in two main groups with respect to color, namely, those with solid-colored seed coats (fig. 20) and those with patterned seed coats (fig. 21). All color types of beans may have

eye markings. The "eye" as referred to in these descriptions is that small colored area surrounding the hilum and above the caruncle, as shown in Figure 22-4. Eyed varieties frequently have a narrow ring immediately surrounding the hilum. This is referred to as the hilum ring. Eyes may be patterned, as in Old Fashioned Yellow Eye (fig. 22-1), in which the eye is defined by an outer snuffbrown ring. At each end of the hilum there is a Roman-ocher colored area, and at each side of the hilum there is a white area which frequently connects up with the white body color of the seed coat. Color patterns are distributed throughout the entire seed coat or are localized in an area of varying size surrounding the eye. Beans with fully patterned seed coats are of three types, namely, those showing longitudinal stripes (fig. 21-1), those showing less definitely defined color areas (fig. 21-4), and those with definitely defined

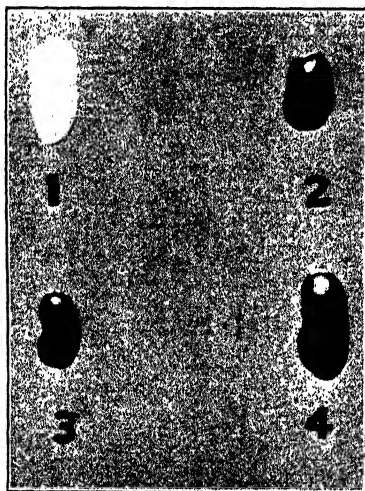


FIGURE 20.—Solid-colored seed coats of beans of the following varieties: 1, White Kidney; 2, Brown Swedish; 3, Black Turtle Soup; 4, Red Kidney

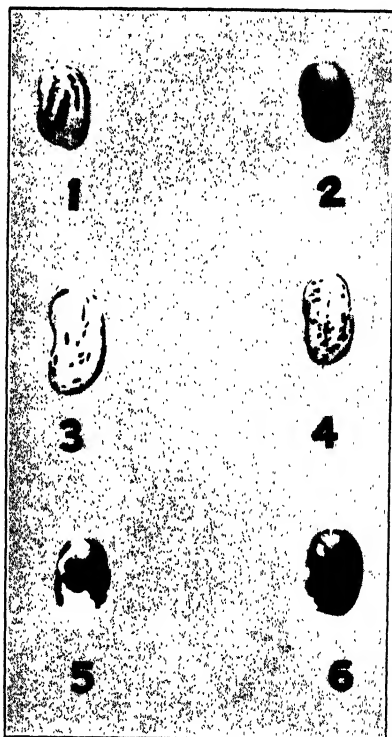


FIGURE 21.—Patterned seed coats of beans of the following varieties: 1, Zebra; 2, Hansen; 3, Improved Goddard; 4, Selection 148; 5-6, Mottled Red Indian

color areas (fig. 21-5). Beans with color patterns surrounding the eye are of two types those showing one color (fig. 22, 1 and 2) and those showing two colors.

The margins of the patterns surrounding the eye vary in extent and in definiteness. (Fig. 22.) All color determinations as herein reported were made by comparing fresh, fully mature, dry seed with the colors of a standard chart (12). In a study of garden beans Shaw and Norton (11) point out that there is no consistent correlation between color of seed coat and color of flowers, which is in accord with the findings of this study of field beans.

SUMMARY OF CHARACTERS USEFUL IN CLASSIFYING FIELD BEANS

1. The glabrous leaf character serves to distinguish *Phaseolus lunatus* and *P. acutifolius* A. Gray var. *latifolius* G. F. Freeman from *P. multiflorus* Willd. and *P. vulgaris* L.
2. Radiating lines from the hilum and shape and size of seed permit differentiation between *P. lunatus* and *P. acutifolius* var. *latifolius*.
3. Tuberous roots characterize *P. multiflorus* and a taproot with branches characterizes *P. vulgaris*.
4. The seedlings of *P. multiflorus* do not carry the cotyledons aboveground, whereas the seedlings of *vulgaris* do.
5. The following characters may be used in classifying varieties of the common field bean:
 - a. Habit of growth of the plants, bush or trailing.
 - b. Number and length of internodes.
 - c. Character of leaf surface.
 - d. Color of flowers.
 - e. Time of maturity.
 - f. Pod shape, texture, and color.
 - g. Pod dimensions, width and length.
 - h. Position, length, and shape of spur.
 - i. Dry-seed characters — shape, size, color of seed coat, presence or absence of eye markings, and color of eye.
6. The following vegetative characters may be used in describing varieties:
 - a. Size, shape, color, and character of surface of seedling leaves.
 - b. Color of mature leaves and stems.
 - c. Size and shape of mature leaves.

CLASSIFICATION AND DESCRIPTION OF FOUR OF THE ECONOMIC SPECIES OF THE GENUS PHASEOLUS

The varieties of beans grown in the United States for human food either in the form of immature pods containing the partially developed seeds, for shell beans or for the dry seed, are very largely included in four species of the genus *Phaseolus*. The four species may be classified as follows:

- A. Leaves glabrous.
 - B. Seeds with conspicuous lines radiating from the hilum to the dorsal region; perennial in Tropics. *Phaseolus lunatus* L. Lima bean.
 - BB. Seeds without conspicuous lines radiating from the hilum to the dorsal region. *Phaseolus acutifolius* A. Gray var. *latifolius* G. F. Freeman. Tepary bean.
- AA. Leaves pubescent.
 - B. Roots tuberous, often perennial in warm climates, cotyledons not raised above the ground in the seedlings. *Phaseolus multiflorus*. Runner bean.
 - BB. Roots not tuberous, annuals, cotyledons raised above the ground in the seedlings. *Phaseolus vulgaris* L. Common bean.

The following descriptions are largely from the Standard Cyclopedia of Horticulture by Bailey (2).

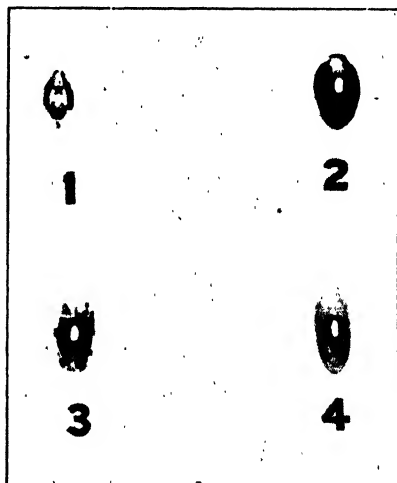


FIGURE 22.—Eyes of beans of the following varieties: 1, Patterned eye of Old Fashioned Yellow Eye; 2, Improved Yellow Eye; 3, mottled eye pattern of China Red Eye; 4, Long Yellow

THE LIMA BEAN

The Lima bean is of two main types, in both of which occur dwarf or bush forms. Sieva type has slender stems, thin, short, broad, ovate, pointed leaflets except in the willow leaf forms. Flowers greenish white. Pods small, papery, curved apex ending in distinct spur, seeds small, flat. Relatively early. The Lima type is tall, robust growing, late maturing. Leaflets large and thick. Pods straight without distinct spur, seeds large, flat, or round. Commercial production of the large-seeded type of the Lima bean for the dry seeds is limited in the United States to the southern coast region of California. More Lima beans are produced in California than all the other species combined.

THE TEPARY BEAN

Plants twining, leaflets thin, glabrous, ovate to broadly lanceolate, acuminate. Flowers on peduncles shorter than the leaves, white or pale violet, dry pods papery, flat, straight to slightly curved, with prominent spur. Seeds small, round, oval to nearly round to strongly flattened. A drought-resistant bean adapted to the hot semiarid regions of the Southwest desert region. Forms a small part of the dry beans of commerce.

THE SCARLET RUNNER BEAN

Plants tall, twining, slender, minutely pubescent. Leaflets thin, large, rhombic-ovate, and acute. Flowers on long racemes, pods long with distinct apex, seeds large, much flattened to nearly cylindrical. The Scarlet Runner, with red flowers and colored seeds, and the Dutch Case Knife, with white flowers and seeds, are the two most widely grown varieties of this species.

THE COMMON BEAN

Plants both bush and trailing forms. Leaves and stems pubescent. Leaflets pubescent, rhombic-ovate or ovate in shape, peduncles shorter than the petioles, few flowered. Flowers white, yellowish, or greenish white or lilac. Pods straight or distinctly curved, ending in a distinct spur. Immature pods of snap beans either yellow or green in color, those of field varieties green.

The field varieties of the common bean are distinguished by their fibrous pods from the snap beans, which have very little or no fiber in the pods.

KEY TO THE VARIETIES OF PHASEOLUS VULGARIS

- A. Seeds white.
 - B. Plants bush.
 - C. Seeds reniform to subreniform.
 - D. Seeds flat, thickness less than 0.7 of width. p. 29..... French Dwarf Kidney
 - DD. Seeds round, thickness at least 0.8 of width. p. 29..... White Kidney
 - CC. Seeds not reniform or subreniform.
 - D. Seeds round, thickness at least 0.8 of width, small, conspicuously veined. p. 29..... Selection 90.
 - BB. Plants trailing.
 - C. Seeds reniform to subreniform.
 - D. Seeds flat, thickness less than 0.7 of width.
 - E. Pods wide, over 1.1 cm.
 - F. Pods with uniform curve. p. 29..... Haricot.
 - FF. Pods curved at free and reflexed at stem end. p. 29..... Selection No. 162.
 - EE. Pods intermediate in width, 1.1 cm or less.
 - F. Pods distinctly curved.
 - G. Average width pod 1 cm. p. 30..... Great Northern
 - GG. Average width pod 1.1 cm. p. 32..... Selection 97.
 - FF. Pods slightly curved. p. 32..... Selection 85.
 - DD. Seeds intermediate, thickness 0.7 to 0.8 of width.
 - E. Pods flat to intermediate, curved.
 - F. Average pod width less than 1 cm. p. 32..... Lady Washington.
 - FF. Average pod width 1 cm or over. p. 32..... Selection 106.

- EE. Pods round, curved.
 - F. Seeds not truncated, medium in size. p. 32. -- Pilot.
 - FF. Seeds truncated, small. p. 32. ----- Yankce Winter.
- DDD. Seeds round, thickness at least 0.8 width. p. 40. ----- California Wonder.
- CC. Seeds not reniform or subreniform.
 - D. Seeds flat, thickness less than 0.7 of width.
 - E. Pods broad. p. 40. ----- Pearce Improved.
 - DD. Seeds intermediate, thickness 0.7 to 0.8 width.
 - F. Pods straight, broad.
 - F. Average thickness of seed over 0.7 of width.
 - G. Average thickness 0.73 of width. p. 40. ----- Burlingame.
 - GG. Average thickness 0.764 of width. p. 40. ----- Selection 154.
 - FF. Average thickness of seed 0.7 of width or less.
 - G. Average length seeds over 0.9 cm. p. 40. ----- White Wonder.
 - GG. Average length seeds 0.9 cm or less. p. 40. ----- Selection 81.
- EE. Pods curved, broad.
 - F. Pods sharply curved, very broad. p. 40. ----- French White.
 - FF. Pods slightly curved.
 - G. Nodes 18-24. p. 41. ----- White Marrow.
 - GG. Nodes 13-15. p. 41. ----- Selection 100.
- DDD. Seeds round, thickness at least 0.8 of width.
 - E. Seeds large. p. 41. ----- Vineless Marrow.
- EE. Seeds small.
 - F. Pods curved, narrow.
 - G. Early maturing.
 - H. Seeds not truncate. p. 41. ----- Snowflake.
 - HH. Seeds truncate. p. 41. ----- Selection 152.
 - GG. Medium maturing.
 - H. Seeds not distinctly truncate. p. 41. ----- Navy Pea.
 - HH. Seed truncate. p. 41. ----- Robust.
 - GGG. Late maturing.
 - H. Spur short, less than 0.6 cm. p. 42. ----- Selection 111.
 - HH. Spur medium length, between 0.6 and 0.9 cm. p. 42. ----- Selection 112.
 - FF. Pods straight or reflexed, midlong, frequently tinted purple. p. 42. ----- Blue Pod.
- AA. Seeds colored.
- B. Plants determinate in growth, bush form.
- C. Seeds reniform to subreniform.
 - D. Seeds intermediate in thickness 0.7 to 0.8 of width.
 - E. Seeds one color.
 - F. Seeds garnet brown.
 - G. Seeds intermediate. p. 42. ----- Red Kidney.
 - GG. Seeds flat. p. 42. ----- Selection 123.
 - FF. Seeds plum violet. p. 42. ----- Selection 149.
 - FFF. Seeds chamois. p. 42. ----- Long Yellow.
 - EE. Seeds more than one color.
 - F. Seeds flesh color, mottled garnet brown. p. 43. ----- Improved Goddard.
 - DD. Seeds round, thickness greater than 0.8 of width.
 - E. Seeds more than one color.
 - F. Seeds tan, mottled, madder brown. p. 43. ----- Emperor of Russia.

- EE. Seeds one color.
 - F. Seeds Roman ocher.
 - G. Mature pods curved.
 - H. Pods long.
 - I. Seeds eyed.
 - J. Stippled. p. 43--- Brown Norwegian.
 - JJ. Not stippled. p. 43--- Selection 140.
 - II. Seeds without eye. p. 43--- Selection 150.
 - HH. Mature pods short, distinctly curved. p. 43--- Selection 141.
 - GG. Mature pods straight. p. 43--- Selection 161.
 - FF. Seeds Naples Yellow. p. 43--- Selection 156.
- CC. Seeds not reniform or subreniform.
 - D. Seeds round, thickness of at least 0.8 of width.
 - E. Seeds solid color.
 - F. Seeds Naples Yellow. p. 44----- Eureka.
 - FF. Seeds Roman ocher.
 - G. Seeds eyed.
 - H. Seed coat not stippled. p. 44----- Brown Swedish.
 - HH. Seed coat stippled. p. 44----- Selection 135.
 - GG. Seeds not eyed. p. 44----- Selection 145.
 - EE. Seeds more than one color.
 - F. Seeds with irregular ox-blood red pattern at hilum. p. 44----- China Red Eye.
 - FF. Seeds with small irregular yellow ocher pattern at eye. p. 44----- Old Fashioned Yellow Eye.
 - BB. Plants indeterminate in growth, trailing.
 - C. Seeds reniform to subreniform.
 - D. Seeds flat, thickness less than 0.7 width.
 - E. Seeds one color.
 - F. Seeds raw sienna. p. 45.----- Arikara Yellow.
 - FF. Seeds black. p. 45----- Black Turtle Soup.
 - FFF. Seeds plum violet in color.
 - G. Spur medium length, between 0.6 and 0.9 cm p. 45----- Hidatsa Red.
 - GG. Spur long, over 0.9 cm. p. 45----- Selection 128.
 - FFFF. Seeds snuff brown. p. 45----- Selection 147.
 - EE. Seeds more than one color.
 - F. Seeds dull purple lake, stippled. p. 45--- Selection 142.
 - FF. Seeds with longitudinal color bands.
 - G. Seeds smoke gray with black bands. p. 46--- Zebra.
 - GG. Seeds stone colored with vinous mauve bands. p. 46--- Hansen.
 - FFF. Seeds with irregular color pattern.
 - G. Seeds pale yellow flesh, marked with black. p. 46----- Selection 148.
 - GG. Seeds salmon flesh, marked with snuff brown. p. 46----- Garaypata.
 - GGG. Seeds plum violet and white. p. 46----- Mottled Red Indian.
 - DD. Seeds intermediate in thickness, 0.7 to 0.8 of width.
 - E. Seeds one color.
 - F. Seeds raw sienna, small in size. p. 47--- Bayou Chico.
 - FF. Seeds pale pink in color, large in size. p. 47--- Bayou Grande.
 - FFF. Seeds pink in color. p. 47----- Pink.
 - EE. Seeds more than one color.
 - F. Seeds flesh color, mottled sepia. p. 47--- Wild Goose.
 - CC. Seeds not reniform or subreniform.
 - D. Seeds round, thickness over 0.8 of width.
 - E. Seeds more than one color.
 - F. Seeds with large sharply defined yellow ocher eye pattern. p. 47----- Improved Yellow Eye.
 - FF. Seeds flesh color mottled garnet brown. p. 47----- Ruby Horticultural Bush.

DESCRIPTIONS OF VARIETIES OF FIELD BEANS

Seed from the various sources was first grown under the name attached. The names were retained with the types that were true to known variety description. Others were given correct names as they were identified, or, if they were not identified, they are reported here by selection number only. Types selected from certain seed stocks, some of which are probably due to hybridization and therefore not true to any known variety description, are also listed under selection number. Many unidentified types were grown in culture. Since their performance record did not warrant continuation they were discarded. Numbers are carried with the retained varieties for reference.

VARIETIES WITH WHITE SEEDS

All varieties with white seeds have leaves and stems green throughout. Differences in shade of green are mentioned in the descriptions when they are distinct. All the white-seeded varieties used have white flowers.

FRENCH DWARF KIDNEY, No. 115

Plants strictly bush, height 35 cm, nodes 5-6. Leaves large. Mature pods not colored, slightly curved, medium texture, flat. Pod length 10.33 and width 1.16 cm. Seeds per pod 4.9. Spur not marginal, curved, length 1.02 cm. Dry seeds reniform: length 1.299, width 0.70, and thickness 0.476 cm. Thickness 0.678 of width. Seeds flat; 3,664 seeds per 1,000 g. Color, milk white (12, p. 11-1, 2). (Figs. 23-7; 26-4.) Late, low productivity, resistant to anthracnose. From Vilmorin & Co., Paris, France.

WHITE KIDNEY, No. 104

Plants strictly bush, height 45-50 cm, nodes 5-6. Leaves large, broad, long pointed. Mature pods not colored, straight, round, length 12.966 cm, width 0.978 cm. Seeds per pod 4.1. Apex abrupt. Spur not marginal, straight, length 1.464 cm. Dry seeds reniform; length 1.597, width 0.729, thickness 0.555 cm. Thickness 0.760 of width; seeds intermediate; 2,067 seeds per 1,000 g. Color, milk white (12, p. 11-1, 2). (Figs. 23-8; 26-5.) Late, low productivity, resistant to anthracnose. From Minnesota Agricultural Experiment Station. St. Paul, Minn., and several seed houses.

SELECTION 90

Plants strictly bush, height 28-34 cm, nodes 5-6. Leaves medium size, long pointed. Mature pods not colored, distinctly curved, round, length 0.025, width 0.956 cm. Seeds per pod 4.6. Spur, marginal, straight, length 1.244 cm. Dry seeds not reniform, conspicuously veined, length 0.873, width 0.651, thickness 0.563 cm. Thickness 0.864 of width. Seeds spherical; 4,473 seeds per 1,000 g. Color fleshy white (12 p. 9). (Figs. 23-9; 26-6.) Early, low productivity and quality, resistant to anthracnose. From Canada.

HARICOT, No. 103

Plant long trailing, height 75 to 140 cm, nodes 14-18. Leaves large, broad, short pointed. Seedling leaves rough wrinkled. Mature pods not colored, medium texture, curved, flat, length 12.215, width 1.241 cm. Seeds per pod 4.2. Apex elongate. Spur marginal, curved, length 1.348 cm. Dry seeds reniform; length 1.423, width 0.818, thickness 0.480 cm. Thickness 0.587 of width. Seeds flat; 2,597 seeds per 1,000 g. Color milk white (12, p. 11-1, 2). (Figs. 23-10; 26-7.) Very late, medium productivity, resistant to anthracnose.

SELECTION 162

Similar to Haricot; nodes 17-22. Mature pods not colored, curved at free end and reflexed at stem end, medium texture, length 12.53, width 1.397 cm. Apex elongate. Spur marginal, curved, length 1.027 cm. Dry seeds reniform;

length 1.325, width 0.762, thickness 0.483 cm. Thickness 0.633 of width. Seeds flat; 2,584 seeds per 1,000 g. (Figs. 23-11; 26-8.) Very late, low productivity, resistant to anthracnose.

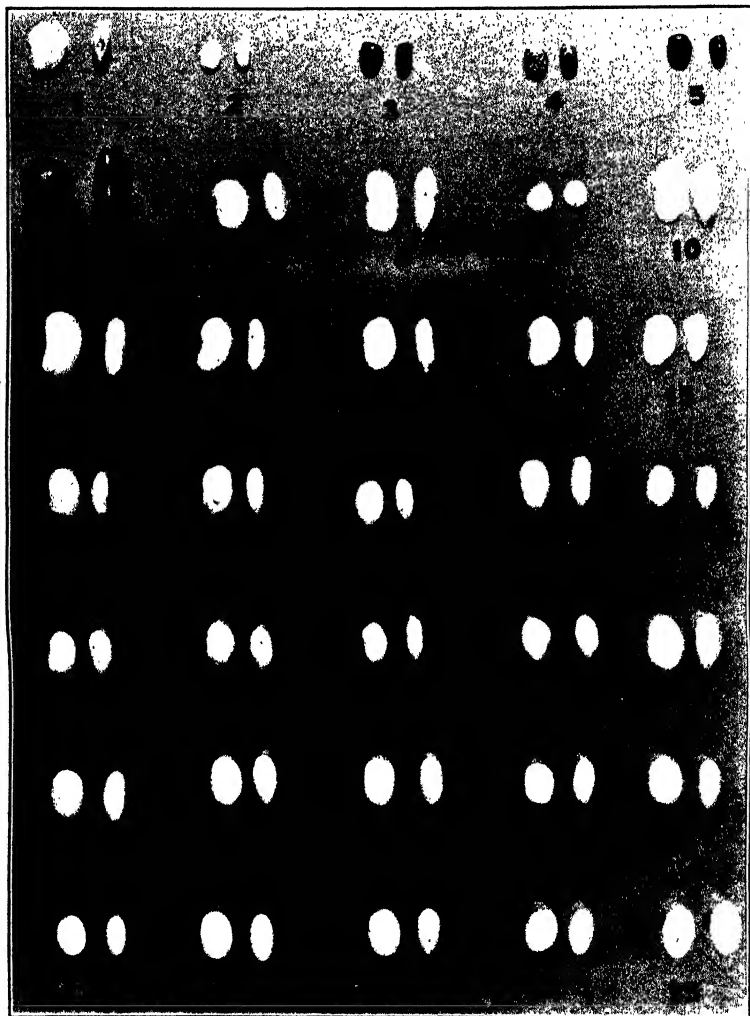


FIGURE 23.—Side and ventral views of ripe seeds: 1, Dwarf Lima; 2, White Tepary; 3, Yellow Tepary; 4, Mottled Tepary; 5, Black Tepary; 6, Scarlet Runner; 7, French Dwarf Kidney, No. 115; 8, White Kidney, No. 104; 9, Selection 90; 10, Haricot, No. 103; 11, Selection 162; 12, Great Northern, No. 78; 13, Selection 97; 14, Selection 86; 15, Selection 163; 16, Selection 85; 17, Selection 166; 18, Lady Washington, No. 82; 19, Selection 106; 20, Selection 89; 21, Selection 164; 22, Pilot, No. 83; 23, Yankee Winter, No. 107; 24, California Wonder, No. 91; 25, Pearce Improved, No. 70; 26, Burlingame, No. 80; 27, Selection 109; 28, Selection 154; 29, Schofield Pea, No. 88; 30, White Wonder, No. 62; 31, Selection 81; 32, French White, No. 98; 33, White Marrow, No. 87; 34, Selection 100; 35, Vineless Marrow, No. 79. $\times \frac{1}{10}$.

GREAT NORTHERN, No. 78

Plant short trailing, height 70.75 cm, nodes 16-20. Leaves medium size, short pointed. Mature pods often splashed purple, distinctly curved, flat,

papery in texture, length 9.295, width 1.013 cm. Seeds per pod 4.8. Apex slightly elongated. Spur marginal, curved, length 0.899 cm. Dry seeds reniform; length 1.325, width 0.715, thickness 0.477 cm. Thickness 0.667 of width.

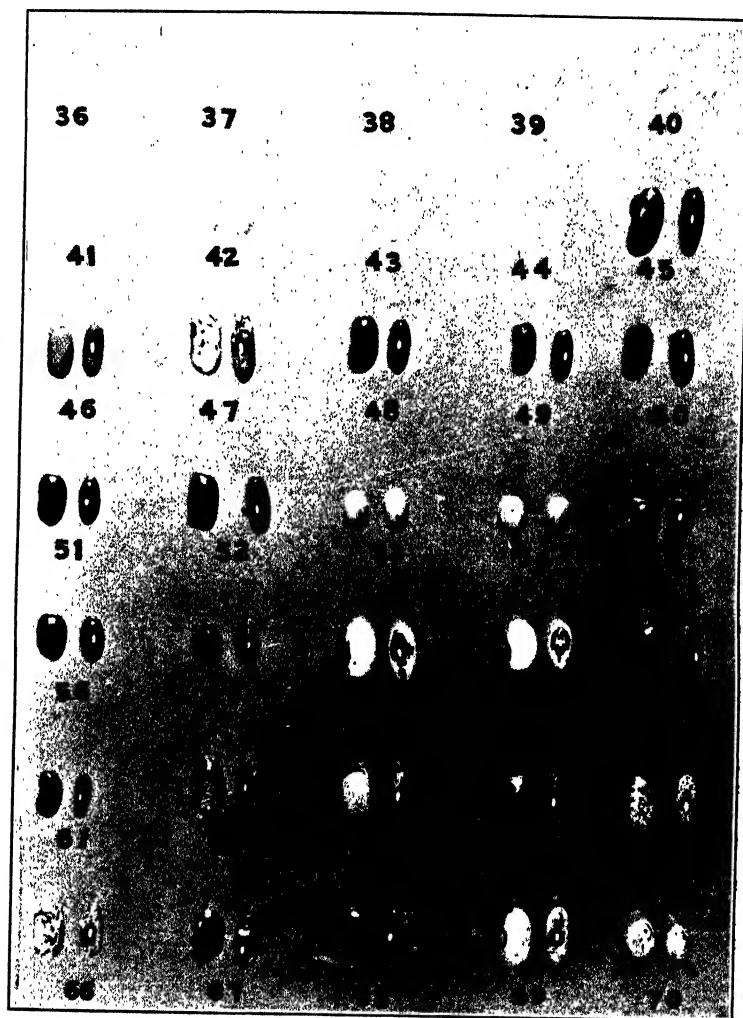


FIGURE 24.—Side and ventral views of ripe seeds: 36, Navy Pea, No. 94; 37, Snowflake, No. 75; 38, Selection 152; 39, Wolverine, No. 110; 40, Robust, No. 76; 41, French Dwarf Rice, No. 101; 42, Selection 112; 43, Selection 111; 44, Blue Pod, No. 102; 45, Red Kidney, No. 116; 46, Long Yellow, No. 124; 47, Improved Goddard, No. 139; 48, Selection 140; 49, Brown Norwegian, No. 133; 50, Selection 150; 51, Selection 141; 52, Selection 161; 53, Selection 156; 54, Eureka, No. 130; 55, Brown Swedish, No. 144; 56, Selection 135; 57, Selection 145; 58, China Red Eye, No. 151; 59, Old Fashioned Yellow Eye, No. 125; 60, Arikara Yellow, No. 117; 61, Black Turtle Soup, No. 118; 62, Selection 142; 63, Zebra, No. 129; 64, Hansen, No. 122; 65, Selection 148; 66, Garaypata, No. 127; 67, Mottled Red Indian, No. 157; 68, Bayou Chico, No. 158; 69, Bayou Grande, No. 159; 70, Wild Goose, No. 121. $\times \frac{9}{16}$.

Seeds flat; 3,357 seeds per 1,000 g. Color creamy white (12, p. 10-1, 2). (Figs. 23-12; 27-1.) Veined. Medium late, productive, susceptible to anthracnose. Synonym South Dakota Improved.

SELECTION 97

Similar to Great Northern. Pods more curved, length 11.05, width 1.33 cm. Seeds per pod 5.2. Dry seeds, length 1.235, width 0.706, thickness 0.480 cm. Thickness 0.679 of width. Seeds flat. (Figs. 23-13; 27-2.) Received under name of French Dwarf Kidney.

SELECTION 85

Similar to Great Northern. Pods only slightly curved, length 9.464, width 1.015 cm. Seeds per pod 5.1. Dry seeds less reniform, length 1.203, width 0.713, thickness 0.502 cm. Thickness 0.704 of width. Seeds flat. (Figs. 23-16; 27-3.)

LADY WASHINGTON, No. 82

Plant short trailing, height 65-70 cm, nodes 17-20. Leaves dark green, medium size, short pointed. Pods medium texture, not colored, slightly curved, flat, length 9.790, width 0.873 cm. Seeds per pod 5.5. Apex elongated. Spur marginal, curved, length 0.965 cm. Dry seeds subreniform, ends round, length 0.899, width 0.607, thickness 0.430 cm. Thickness 0.708 of width. Seeds flat; 5,544 seeds per 1,000 g. Color milk white (12, p. 11-1, 2). (Figs. 23-18; 27-4.) Late,

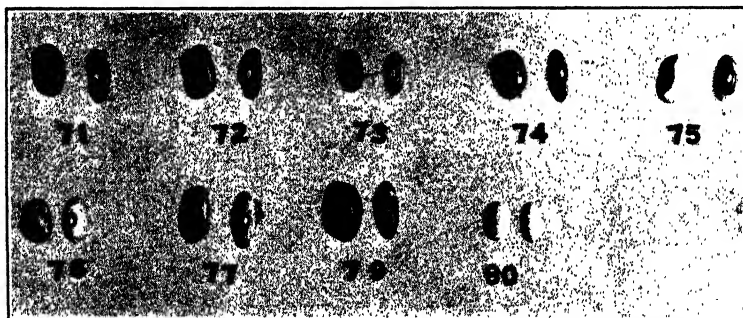


FIGURE 25.—Side and ventral views of ripe seeds: 71, Fidatsa Red, No. 131; 72, Selection 128; 73, Selection 136; 74, Selection 147; 75, Improved Yellow Eye, No. 120; 76, Ruby Horticultural Bush, No. 129; 77, Selection 143; 78, Pink, No. 160; 79, Small White, No. 108. $\times 91a$.

medium productivity, resistant to anthracnose. Not well adapted to this section. From Germaine Seed Co., Los Angeles, Calif.

SELECTION 106

Similar to Lady Washington. Mature pods, length 10.968, width 1.067 cm. Seeds per pod 5.0. Apex abrupt. Spur marginal, curved, length 0.833 cm. Dry seeds, length 1.164, width 0.680, thickness 0.505 cm. Thickness 0.741 of width; 3,494 seeds per 1,000 g. (Figs. 23-19; 27-5.) Productive. Susceptible to anthracnose. Received under the name of Small White.

PILOT, No. 83

Plant short trailing, height 60-65, cm, nodes 16-18. Leaves medium size, short pointed, dark green. Mature pods not colored, curved, length 9.585, width 1.475 cm. Apex abrupt. Spur not marginal, curved, length 1.437 cm. Seeds per pod 5.2. Dry seeds, length 0.824, width 0.617, thickness 0.512 cm. Thickness 0.830 of width. Seeds round; 4,237 seeds per 1,000 g. Color milk white (12, p. 11-1, 2). (Fig. 23-22.) Productive, resistant to anthracnose. From Will Seed Co., Bismarck, N. Dak.

YANKEE WINTER, No. 107

Plant short trailing, height 65-75 cm, nodes 18-22. Leaves small, short pointed. Mature pods not colored, slightly curved, round, medium texture, ventral suture depressed, length 8.948, width 0.762 cm. Apex slightly elongated. Spur marginal,

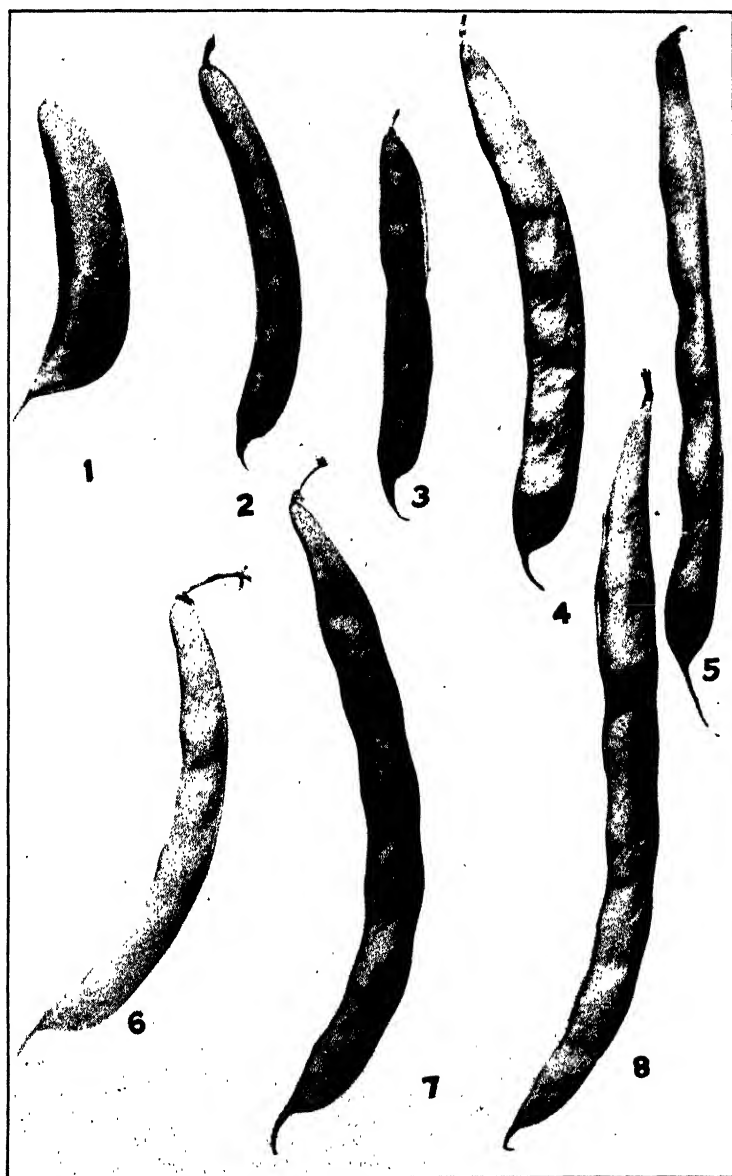


FIGURE 26.—Side view of mature pods: 1, Dwarf Lima; 2, White Tepary; 3, Yellow Tepary; 4, French Dwarf Kidney, No. 115; 5, White Kidney, No. 104; 6, Selection 90; 7, Haricoto, No. 103; 8, Selection 162. $\times \frac{3}{10}$

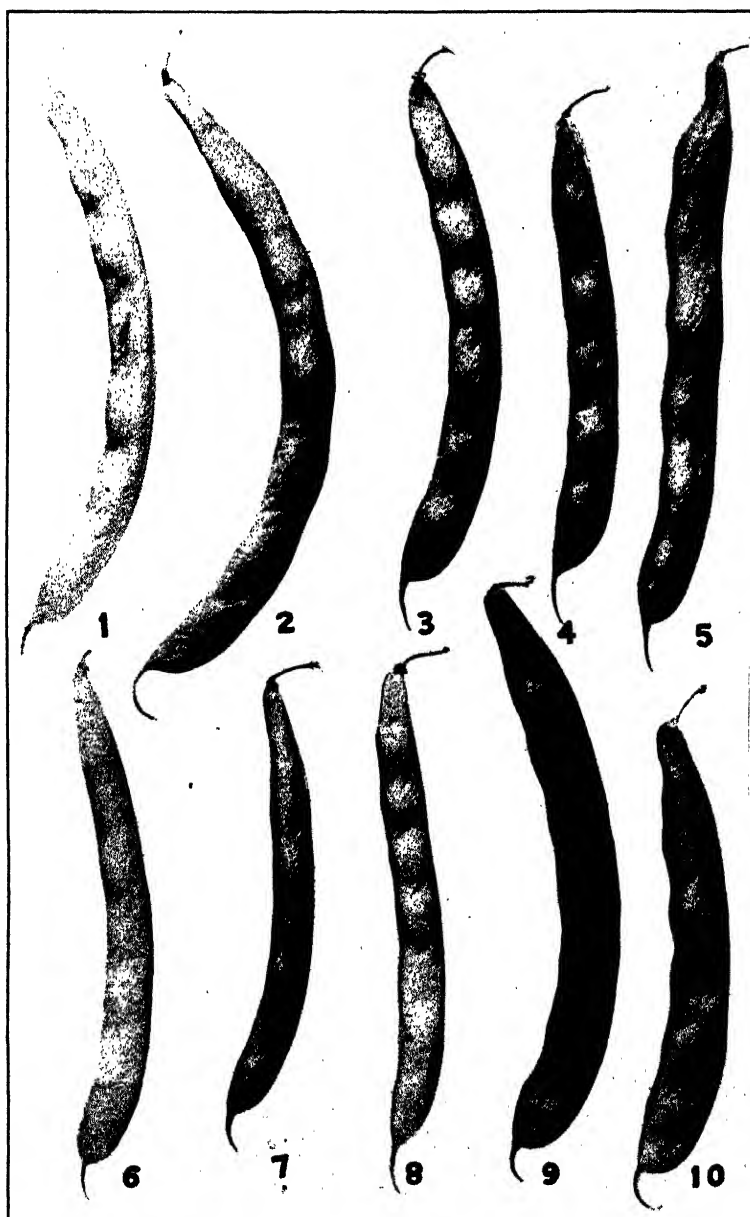


FIGURE 27.—Side view of mature pods: 1, Great Northern, No. 78; 2, Selection 97; 3, Selection 85; 4, Lady Washington, No. 82; 5, Selection 106; 6, Selection 164; 7, Yankee Winter, No. 107; 8, California Wonder, No. 91; 9, Pearce Improved, No. 70; 10, Burlingame, No. 80. $\times \frac{7}{10}$

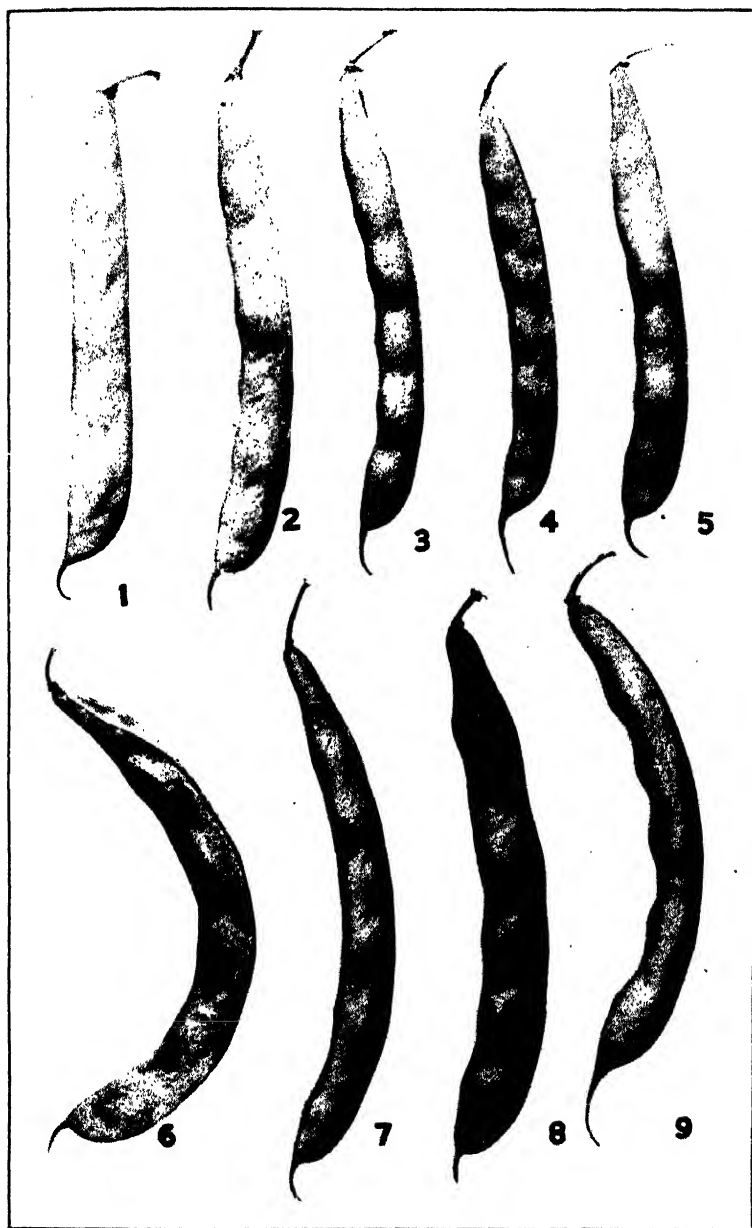


FIGURE 28.—Side view of mature pods: 1, Selection 109; 2, Selection 154; 3, Schofield Pea, No. 88; 4, Selection 81; 5, White Wonder, No. 92; 6, French White, No. 98; 7, White Marrow, No. 87; 8, Selection 100; 9, Vineless Marrow, No. 79. $\times \frac{3}{4}$

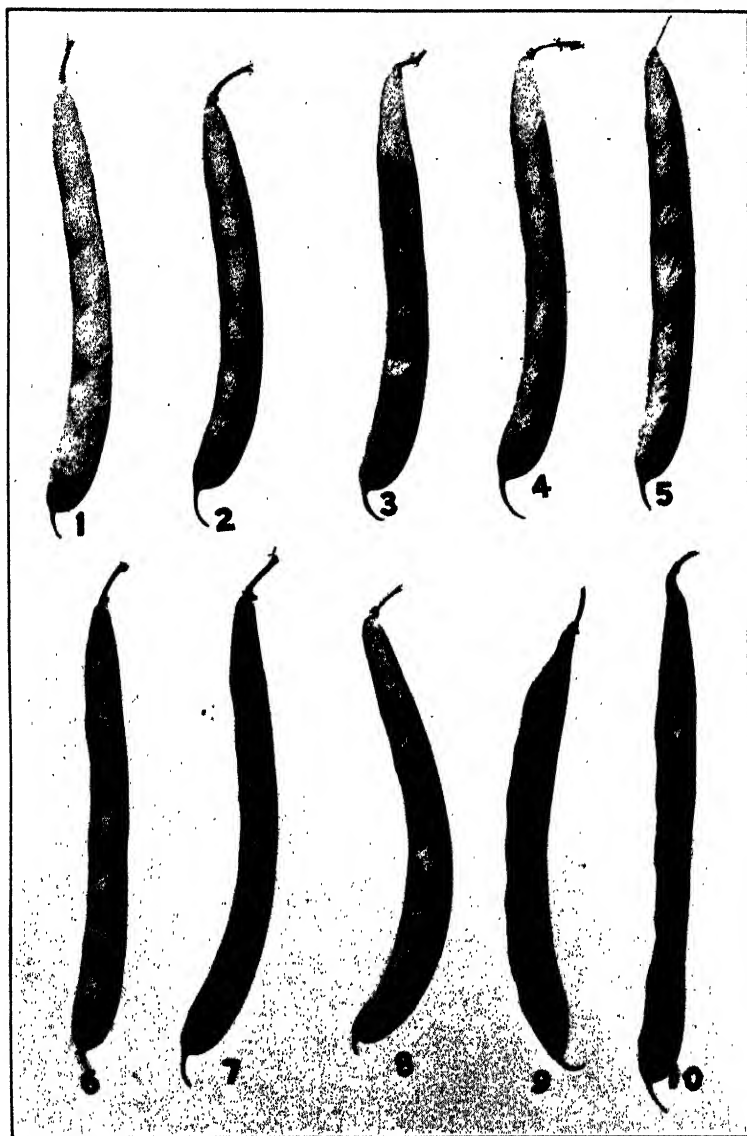


FIGURE 29.—Side view of mature pods: 1, Navy Pea, No. 94; 2, Snowflake, No. 75; 3, Selection 152; 4, Wolverine, No. 110; 5, Robust, No. 76; 6, French Dwarf Rice, No. 101; 7, Selection 112; 8, Selection 111; 9 and 10, Blue Pod, No. 102. $\times \frac{1}{10}$

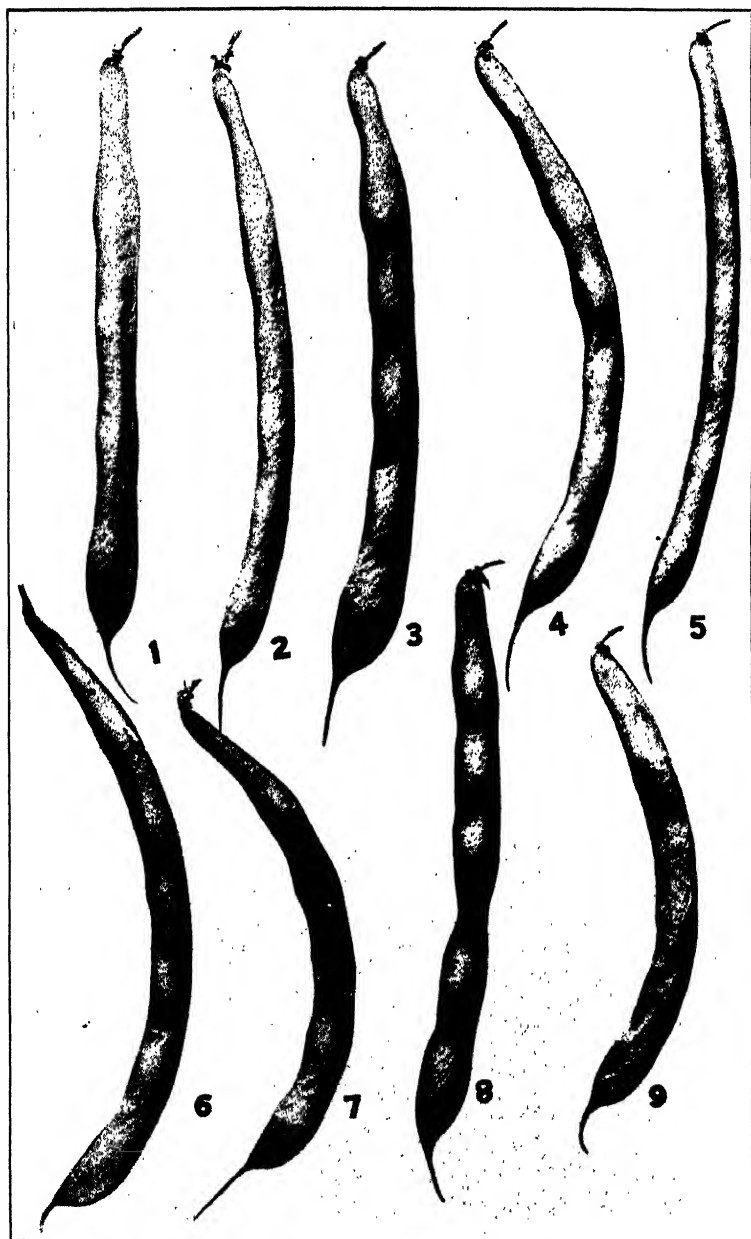


FIGURE 30.—Side view of mature pods: 1, Red Kidney, No. 116; 2, Long Yellow, No. 124; 3, Improved Goddard, No. 139; 4, Selection 140; 5, Brown Norwegian, No. 133; 6, Selection 150; 7, Selection 141; 8, Selection 161; 9, Selection 155. $\times \frac{3}{10}$

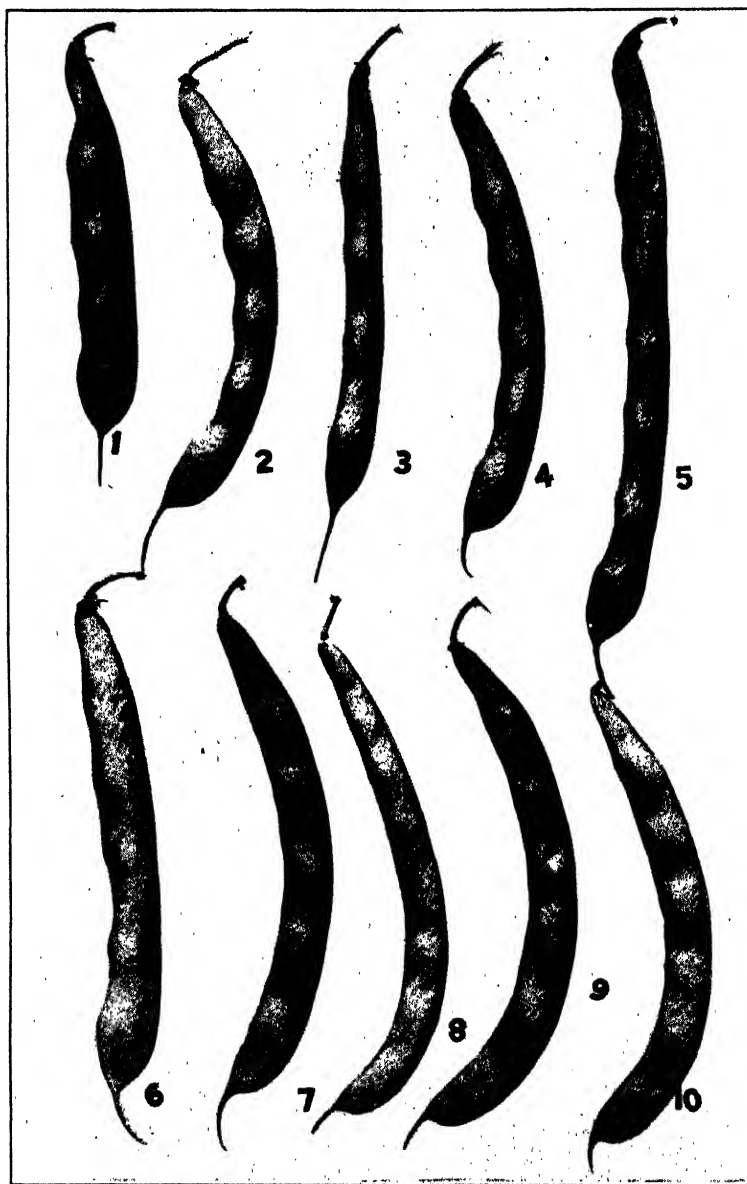


FIGURE 31.—Side view of mature pods: 1, Eureka, No. 130; 2, Brown Swedish, No. 144; 3, Selection 135; 4, Selection 145; 5, China Red Eye, No. 151; 6, Old-Fashioned Yellow Eye, No. 125; 7, Arikara Yellow, 117; 8, Black Turtle Soup, No. 118; 9, Selection 142; 10, Zebra, No. 126. $\times \frac{3}{10}$

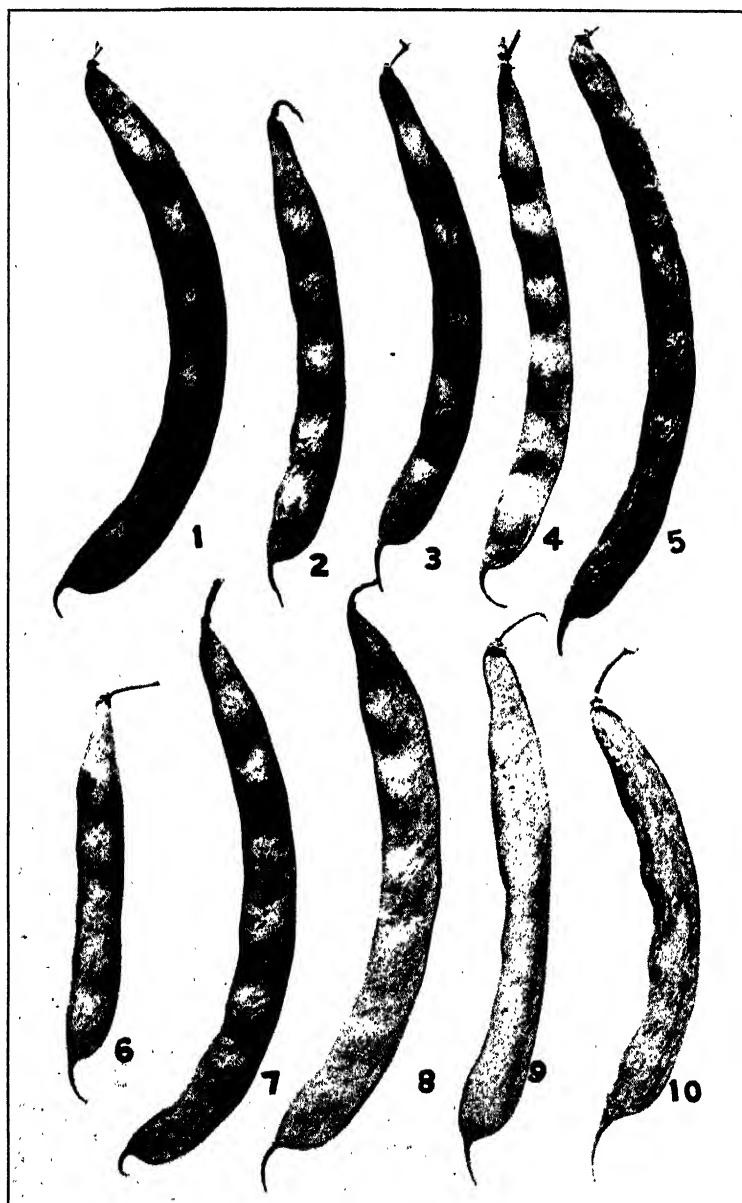


FIGURE 32.—Side view of mature pods: 1, Hansen, No. 122; 2, Selection 148; 3, Garaypata, No. 127; 4, Bayou Chico, No. 168; 5, Bayou Grande, No. 159; 6, Wild Goose, No. 121; 7, Hidatsa Red, No. 131; 8, Selection 147; 9, Improved Yellow Eye, No. 120; 10, Ruby Horticultural Bush, No. 129. $\times \frac{1}{10}$

curved, length 0.719 cm. Seeds per pod 6.2. Dry seeds reniform, ends truncate, length 0.844, width 0.554, thickness 0.429 cm. Thickness 0.762 of width. Seeds intermediate; 6,644 seeds per 1,000 g. Color milk white (12, p. 11-1, 2). (Figs. 23-23; 27-7.) Very late, low productivity, susceptible to anthracnose. From an exhibit at the Minnesota State fair.

CALIFORNIA WONDER, No. 91

Plant short trailing, height 90-120 cm, nodes 18-24. Leaves small, short pointed. Mature pods not colored, medium texture, curved, length 9.570, width 0.887 cm. Seeds per pod 5.9. Apex slightly elongated. Spur marginal, curved, length 0.808 cm. Dry seeds subreniform, ends round, length 0.938, width 0.634, thickness 0.513 cm. Thickness 0.808 of width. Seeds round; 4,905 seeds per 1,000 g. Color milk white (12, p. 11-3, 4). (Figs. 23-24; 27-8.) Late, medium productive, susceptible to anthracnose. From Northrup, King & Co., Minneapolis, Minn.

PEARCE IMPROVED, No. 70

Plant short trailing, height 80 to 110 cm, nodes 16-22. Leaves medium size, short pointed. Mature pods not colored, thin, very flat, curved, length 10.275, width 1.23 cm. Seeds per pod 4.2. Apex abrupt. Spur curved, marginal, average length 1.098 cm. Dry seeds not reniform, ends truncated, length 1.176, width 0.761, thickness 0.494 cm. Thickness 0.649 of width. Seeds flat; 3,185 seeds per 1,000 g. Color milk white (12, p. 11-1, 2). (Figs. 23-25; 27-9.) Very late, productive, susceptible to anthracnose. From Ontario Agricultural College, Guelph, Ontario.

BURLINGAME, No. 80

Plant short trailing, height 80-90 cm, nodes 15-16. Leaves medium size, short pointed. Mature pods not colored, medium texture, straight, flat, length 10.146, width 1.163 cm. Seeds per pod 4.5. Spur marginal, curved, length 1.151 cm. Dry seeds not reniform, ends truncated, length 1.087, width 0.735, thickness 0.538 cm. Thickness 0.730 of width. Seeds intermediate; 3,044 seeds per 1,000 g. Color milk white (12, p. 11-1, 2). (Figs. 23-26; 27-10.) Medium late, productive, susceptible to anthracnose. Synonyms Medium and Schofield Pea. From several seedsmen.

SELECTION 154

Similar to Burlingame. Mature pods slightly curved, less flattened. Length 9.904, width 1.127 cm. Apex abrupt. Spur marginal, length 1.179. Dry seed, length 1.058, width 0.750, thickness 0.570 cm. Thickness 0.764 of width. Seeds intermediate. (Figs 23-28; 28-2.) From Ontario Agricultural College, Guelph, Ontario, under name of Medium.

WHITE WONDER, No. 92

Similar to Burlingame. Dry pods. Length 0.739, width 1.127 cm. Seeds per pod 3.9. Spur length 1.034 cm. Dry seeds, length 1.042, width 0.702, thickness 0.493 cm. Thickness 0.702 of width. Seeds flat. (Figs. 23-30; 28-5.)

SELECTION 81

Similar to White Wonder but shorter pod and shorter, flatter seed. Dry seed, length 0.898, width 0.670, thickness 0.457 cm. Thickness 0.680 of width. Seeds flat. (Figs. 23-31; 28-4.)

FRENCH WHITE, No. 98

Plant short trailing, height 95 cm, nodes 16-18. Leaves medium size, short pointed. Mature pods not colored, thin, decidedly curved, very flat, length 11.767, width 1.297 cm. Seeds per pod 5.6. Apex abrupt. Spur not marginal, curved, length 1.028 cm. Dry seeds not reniform, ends round, length 1.113, width 0.740, thickness 0.569 cm. Thickness 0.769 of width. Seeds intermediate; 3,190 seeds per 1,000 g. Color milk white (12, p. 11-1, 2). (Figs. 23-32; 28-6.) Low productivity. From A. H. Huchting, Oceanside, Calif.

WHITE MARROW, No. 87

Plant long trailing, height 135-145 cm, nodes 18-24. Leaves large, long pointed. Mature pods not colored, medium texture, slightly curved, flat, length 10.719, width 1.079 cm. Seeds per pod 5.8. Spur not marginal, curved, length 0.905 cm. Dry seeds not reniform, ends round, length 1.021, width 0.697, thickness 0.560 cm. Thickness 0.809 of width. Seeds round; 3,827 seeds per 1,000 g. Color milk white (12, p. 11-1, 2). (Figs. 23-33; 28-7.) Very late, productive, susceptible to anthracnose. From several seedsmen.

SELECTION 100

Similar to White Marrow, but lower growing, 90 cm tall, and smaller number of nodes, 13-15. Dry-pod length 10.332, width 1.244 cm. Spur length 0.994 cm. Seeds per pod 5.5. Dry seeds, length 1.013, width 0.719, thickness 0.582 cm. Thickness 0.809 of width. (Figs. 23-34; 28-8.)

VINELESS MARROW, No. 79

Plant short trailing, height 80 cm, nodes 12-16. Seedling leaves wrinkled. Leaves large, broad, long pointed. Mature pods not colored, coarse, curved, round, length 10.539, width 1.104 cm. Seeds per pod 4. Apex elongated. Spur not marginal, straight to slightly curved, length 1.431 cm. Dry seeds not reniform, ends round, length 1.129, width 0.762, thickness 0.676 cm. Thickness 0.887 of width. Seeds round and seed coat often wrinkled; 2,180 seeds per 1,000 g. Color milk white (12, p. 11-3, 4). (Figs. 23-35; 28-9.) Late, productive, very susceptible to anthracnose. From Ontario Agricultural College, Guelph, Ontario, and from several seedsmen.

SNOWFLAKE, No. 75

Plant short trailing, erect, height 75-80 cm, nodes 16-20. Leaves small, short pointed. Mature pods not colored, medium in texture, slightly curved, length 8.497, width 0.886 cm. Spur not marginal, length 0.804 cm. Seeds per pod 5.3. Dry seeds not reniform, ends round, length 0.826, width 0.62, thickness 0.519 cm. Thickness 0.837 of width. Seeds round. Color milk white (12, p. 11-1, 2). (Figs. 24-37; 29-2.) Early maturing, productive. From Gregory & Sons, Boston, Mass., and from several other sources. Wolverine is similar with slightly more globose seeds. (Figs. 24-39; 29-4.)

SELECTION 152

Similar to Snowflake. Pods round, length 8.805, width 0.785 cm. Seeds per pod 5.7. Spur length 0.846 cm. Dry seeds truncated, length 0.818, width 0.556, thickness 0.471 cm. Thickness 0.832 of width. Seed coat fleshy white (12, p. 9), veined. (Figs. 24-38; 29-3.) Early maturing, moderately productive. From a farm in Washington County, Minn., on which it had been grown for a number of years.

NAVY PEA, No. 94

Plant short trailing, much branched, erect, height 75-80 cm, nodes 16-20. Leaves small, short pointed. Mature pods not colored, medium texture, slightly curved, length 8.644, width 0.882 cm. Seeds per pod 4.9. Apex abrupt. Spur not marginal, curved, length 0.819 cm. Dry seeds not reniform, slightly truncated, length 0.813, width 0.605, thickness 0.523 cm. Thickness 0.864 of width. Seeds round; 4,905 seeds per 1,000 g. Color milk white (12, p. 11-1, 2). (Fig. 24-36; 29-1.) Medium early maturing, productive, resistant to anthracnose and blight. From Northrup, King & Co. of Minneapolis, Minn. French Dwarf Rice, No. 101 is similar to Navy Pea. (Figs. 24-41; 29-6.)

ROBUST, No. 76

Similar to Navy Pea. Seeds more truncated and conspicuously veined. Pod measurements, length 8.895, width 0.886 cm. Spur length 0.810 cm. Seeds per pod 5.7. Dry seeds, length 0.844, width 0.600, thickness 0.499 cm. Thickness 0.832 of width. Seeds round. Color fleshy white (12, p. 9). Medium early maturing, very productive, resistant to anthracnose and blight. Mosaic not observed on plants. (Figs. 24-40; 29-5.)

SELECTION 111

Similar to Navy Pea but distinct in having shorter spur. Spur length 0.507 cm. Pod measurements, length 8.335, width 0.90 cm. Seeds per pod 4.9. Dry seeds, length 0.848, width 0.633, thickness 0.526 cm. Thickness 0.831 of width. Seeds round. Late maturity. Medium productivity. (Figs. 24-43; 29-8.)

SELECTION 112

Similar to Navy Pea but later in maturity. Pod measurements, length 8.49, width 0.820 cm. Spur length 0.786 cm. Seeds per pod 5. Dry-seed measurements, length 0.817, width 0.577, thickness 0.491 cm. Thickness 0.849 of width. Seeds round. Color of seeds fleshy white (12, p. 9). (Figs. 24-42; 29-7.) Late maturity.

BLUE POD, No. 102

Plant short trailing, much branched, erect, height 110 cm, nodes 17-20. Leaves small, short pointed. Mature pods often tinged purple, straight to reflex curved, medium texture, round, length 9.430, width 0.768 cm. Seeds per pod 6.5. Apex abrupt. Spur not marginal, curved, length 0.762 cm. Dry seeds oval, ends round, length 0.807, width 0.553, thickness 0.451 cm. Thickness 0.816 of width. Seeds round, 7,158 seeds per 1,000 g. Color fleshy white (12, p. 9) veined. (Figs. 24-44; 29-9 and 10.) Very late, productive, resistant to anthracnose. From Lompoc Produce Co., Lompoc, Calif.

VARIETIES WITH COLORED SEEDS

RED KIDNEY, No. 116

Plants strictly bush, height 46-50 cm, nodes 6 to 7. Leaves and stems green throughout. Leaves large, long pointed. Flowers, standard lilac (12, p. 176, 1-2). Mature pods often splashed red, coarse, straight, length 12.572, width 1.064 cm. Seeds per pod 4.2. Apex slightly elongated. Spur not marginal, straight, length 1.241 cm. Dry seeds reniform, ends round, length 1.563, width 0.725, thickness 0.567 cm. Thickness 0.782 of width. Seeds intermediate; 2,539 seeds per 1,000 g. Color garnet brown (12, p. 164). (Figs. 24-45; 30-1.) Late, medium productivity, anthracnose resistant, blight susceptible. From New York (Cornell) Agricultural Experiment Station, Ithaca, N. Y.

SELECTION 123

Similar to Red Kidney but seeds flat. Mature pods, length 12.119, width 1.061 cm. Seeds per pod 3.9. Spur length 1.309 cm. Dry seeds, length 1.590, width 0.782, thickness 0.516 cm. Thickness 0.659 of width; 2,193 seeds per 1,000 g.

DARK RED KIDNEY, No. 149

Similar to Red Kidney but seeds plum violet (12, p. 172, 4) and earlier in maturity. Pod measurements, length 12.27, width 1.154 cm. Spur length 1.290 cm. Seeds per pod 3.9. Seed length 1.573, width 0.761, thickness 0.568 cm. Thickness 0.747 of width. Susceptible to anthracnose. From Michigan State College of Agriculture and Agricultural Experiment Station.

LONG YELLOW, No. 124

Plants strictly bush, height 44-46 cm, nodes 5-6. Leaves and stems green throughout. Leaves large, long pointed. Flowers, standard lilac (12, p. 176-3, 4), wings lilac (12, p. 176-1, 2). Mature pods not colored, coarse, slightly curved, length 12.405, width 0.908 cm. Seeds per pod 4.9. Apex slightly elongated. Spur not marginal, straight, length 1.363 cm. Dry seeds, subreniform; length 1.387, width 0.706, thickness 0.537 cm. Thickness 0.760 of width; 2,757 seeds per 1,000 g. Seed color chamois (12, p. 325-1, 2), eye color raw umber (12, p. 301), hilum ring Roman ocher (12, p. 327). (Figs. 24-46; 30-2.) Very early, medium productivity, resistant to anthracnose. From New York (Cornell) Agricultural Experiment Station, Ithaca, N. Y.

IMPROVED GODDARD, No. 139

Plants strictly bush, height 42-44 cm, nodes 6-7. Leaves and stems green throughout. Leaves large, long pointed. Flowers, standard lilac (12, p. 176-3, 4), wings lilac (12, p. 176-1, 2). Mature pods splashed red, coarse, slightly curved, length 13.116, width 1.051 cm. Seeds per pod 4.1. Spur marginal, straight, length 1.572 cm. Dry seeds reniform, ends round, length 1.583, width 0.798, thickness 0.554. Thickness 0.694 of width. Seeds flat. 2,863 seeds per 1,000 g. Color flesh (12, p. 67-1) mottled garnet brown (12, p. 164-4), eye Roman ocher (12, p. 327). No distinct hilum ring. (Figs. 24-47; 30-3.) Very early, medium productivity, anthracnose resistant, blight susceptible. From New York (Cornell) Agricultural Experiment Station, Ithaca, N. Y.

EMPEROR OF RUSSIA, No. 111

Plant strictly bush. Pod measurements, length 12.034, width 0.781 cm. Spur length 1.415 cm. Seed per pod 5.2. Seeds reniform, length 1.415, width 0.620, thickness 0.539 cm. Thickness 0.869 of width. Seeds round. Color of seeds tan (12, p. 317-1, 2) heavily mottled madder brown (12, p. 334, 1-2). From Sweden.

BROWN NORWEGIAN, No. 133

Plants strictly bush, height 40-42 cm, nodes 5-6. Leaves and stems green throughout. Leaves large, long pointed. Flowers, standard lilac (12, p. 176-3, 4) wings lilac (12, p. 176-1, 2). Mature pods not colored, coarse, dehisce readily, slightly curved, round, length 12.267, width 0.900 cm. Seed per pod 4.5. Spur marginal, length 1.37 cm. Dry seeds reniform, ends round, length 1.389, width 0.678, thickness 0.548 cm. Thickness 0.807 of width. 3,047 seeds per 1,000 g. Color, Roman ocher (12, p. 327 3, 4), stippled dark brown, eye snuff brown (12, p. 303), hilum ring Roman ocher (12, p. 327). (Figs. 24-49; 30-5.) Early, medium productivity. From Central Experimental Farms, Ottawa, Canada, under Ottawa No. 71.

SELECTION 140

Similar to Brown Norwegian but seeds are not stippled dark brown. Mature pods not colored, coarse, dehisce readily, distinctly curved, length 11.563, width 0.881 cm. Seeds per pod, 4.7. Apex elongated. Spur marginal, length 1.328 cm. Dry seeds reniform, ends round, length 1.339, width 0.681, thickness, 0.561 cm. Thickness 0.822 of width. Seeds round. 2,746 seeds per 1000 g. Color Roman ocher (12, p. 327-3, 4), eye snuff brown (12, p. 303), hilum ring Roman ocher (12, p. 327). (Figs. 24-48; 30-4.) Early, medium productivity, susceptible to anthracnose and blight. From Owosso, Mich.

SELECTION 150

Similar to Selection 140 except seeds without eye markings and pods distinctly sickle-shaped. Pod length 12.540, width 0.865 cm. Seeds per pod, 5.1. Spur length 1.128 cm. Dry seeds, length 1.361, width 0.673, thickness 0.552 cm. Thickness 0.819 of width. (Figs. 24-50; 30-6.)

SELECTION 141

Similar to Selection 140 except pods are shorter, length 9.820, width 0.913 cm. Spur length 1.448. Pods distinctly sickle-shaped. Seeds per pod 4.1. Dry seeds, length 1.228, width 0.687, thickness 0.536 cm. Thickness 0.794 of width; 2,940 seeds per 1000 g. (Figs. 24-51; 30-7.)

SELECTION 161

Similar to Selection 140 except pods are straight. Seeds per pod 5-6. Spur not marginal. Seeds less reniform and eye marking less distinct. (Figs. 24-52; 30-8.)

SELECTION 156

Plants strictly bush, height 35-38 cm, nodes 6-7. Leaves and stems green throughout. Leaves large, dark green. Flowers white. Mature pods not colored, coarse, curved, flattened, length 9.589, width 1.082 cm. Seeds per pod 4.2. Apex slightly elongated. Spur not marginal, curved length 1.235 cm. Dry seeds reniform, ends round, length 1.072, width 0.652, thickness 0.568 cm.

Thickness 0.870 of width. 2,633 seeds per 1,000 g. Color of seed Naples yellow (12, p. 29-1). (Figs. 24-53; 30-9.) Early, low productivity. From Mada-waska, Canada.

EUREKA, No. 130

Plant strictly bush, height 42-44 cm, nodes 6-7. Leaves and stems green throughout. Leaves large oval, blunt pointed, crumpled. Flowers standard lilac (12, p. 176-3, 4), wings lilac (12, p. 176-1, 2). Mature pods not colored, coarse, straight, round, length 8.902, width 1.104 cm. Seeds per pod 4.6. Apex abrupt. Spur not marginal, straight, length 0.852 cm. Dry seeds, length 0.893, width 0.691, thickness 0.611 cm. Thickness 0.884 of width. Seeds spherical; 3,772 seeds per 1,000 g. Color Naples yellow (12, p. 29-1). (Figs. 24-54; 31-1). Medium late, medium productivity, anthracnose and blight resistant. From New York (Cornell) Agricultural Experiment Station, Ithaca, N. Y., and from growers in Washington County, Minn.

BROWN SWEDISH, No. 144

Plant strictly bush, height 40-42 cm, nodes 6-7. Leaves and stems green throughout. Leaves large, long pointed. Flowers, standard lilac (12, p. 176-3, 4), wings lilac (12, p. 176-1, 2). Mature pods not colored, coarse, dehisce readily at maturity. Distinctly curved, round, length 8.902, width 1.104 cm. Seeds per pod 4.6. Apex slightly elongated. Spur not marginal, straight, length 0.852 cm. Dry seeds oval, ends round, length 1.064, width 0.706, thickness 0.609 cm. Thickness 0.862 of width. 3,018 seeds per 1,000 g. Color Roman ocher (12, p. 327-3, 4), eye snuff brown (12, p. 303), hilum ring Roman ocher (12, p. 327). (Figs. 24-55; 31-2.) Early medium productivity, susceptible to anthracnose and blight. From Northrup, King & Co., Minneapolis, Minn.

SELECTION 135

Similar to Brown Swedish, except pods straight with long spur and seeds stippled dark brown. Dry pods, length 10.46, width 0.955 cm. Spur length 1.523. Dry seeds, length 1.312, width 0.700, thickness 0.500 cm. Thickness 0.714 of width. (Figs. 24-56; 31-3.) From Sweden.

SELECTION 145

Similar to Brown Swedish except pods less curved. Dry pods, length 9.177, width 0.906 cm. Spur length 1.07 cm. Dry seeds, length 1.31, width 0.721, thickness, 0.589 cm. Thickness 0.816 of width. Seeds round. (Figs. 24-57; 31-4.)

CHINA RED EYE, No. 151

Plant strictly bush, height 28-30 cm, nodes 5-6. Leaves and stems green throughout. Flowers white. Mature pods not colored. Coarse, straight, length 11.367, width 1.084 cm. Seeds per pod 4.9. Apex slightly elongated. Spur not marginal, curved, length 1.178 cm. Dry seeds oval, length 1.286, width 0.769, thickness 0.630 cm. Thickness 0.922 of width. 2,092 seeds per 1,000 g. Color white with ox-blood red (12, p. 94) irregular mottled pattern around eye covering approximately one-fourth of entire surface of seed. Eye markings lacking. (Figs. 24-58; 31-5.) Very early, low productivity, susceptible to anthracnose and blight. From New York (Cornell) Agriculture Experiment Station, Ithaca, N. Y.

OLD FASHIONED YELLOW EYE, No. 125

Plant strictly bush, height 50-52 cm, nodes 6-7. Leaves and stems green throughout. Flowers white. Mature pods not colored, coarse, slightly curved, length 10.493, width 1.154 cm. Seeds per pod, 4.4. Spur not marginal, curved, length 1.206 cm. Dry seeds, length, 1.23, width 1.799, thickness 0.679 cm. Thickness 0.848 of width. 2,294 seeds per 1,000 g. Color white with irregular yellow ocher (12, p. 326-4), pattern around hilum covering approximately one-fourth of area of seed. Eye snuff brown (2, p. 303) with white spot on each side of hilum. (Figs. 24-59; 31-6.) Medium early, medium productivity, resistant to anthracnose and blight. From various sources.

ARIKARA YELLOW, No. 117

Plant short trailing, decumbent, not highly branched, height 75 to 80 cm, nodes 15-16. Leaves and stems green throughout. Leaves medium size, broad short pointed. Flowers white. Mature pods not colored, papery texture, curved, length 9.695, width 0.990 cm. Seeds per pod, 5.4. Apex abrupt. Spur marginal, curved, length 0.918 cm. Dry seeds reniform; length 1.302, width 0.711, thickness 0.454 cm. Thickness 0.624 of width. Seeds flat; 3,983 seeds per 1,000 g. Color raw sienna (12, p. 329-1, 2). No eye markings. (Figs. 24-60; 31-7.) Medium early, productive, resistant to anthracnose and blight. From Oscar Will Seed Co., Bismarck, N. Dak., under the name given. Also received under name of Yellow Indian. Resembles most Great Northern except in seed color.

BLACK TURTLE SOUP, No. 118

Plant short trailing, erect, height 58-60 cm, nodes 13-14. Leaves medium size, dark green. Stems more or less purple. Flowers, standard magenta (12, p. 182-2), wings violet rose (12, p. 154-2). Mature pods often tinged purple, medium coarse, distinctly curved near free end, length 10.379, width 0.791 cm. Seeds per pod, 5.4. Apex abrupt. Spur marginal, straight, length 1.025 cm. Dry seeds reniform; flat length 1.040, width 0.597, thickness 0.393 cm. Thickness 0.656 of width. Seeds flat; 5,332 seeds per 1,000 g. Color violet black (12, p. 347-4). (Figs. 24-61; 31-8.) Late, very productive, very resistant to anthracnose, susceptible to blight.

HIDATSA RED, No. 131

Plant short trailing, height 75 to 90 cm, nodes 23-26. Leaves and stems green throughout. Leaves large, short pointed, very pubescent. Flowers white. Mature pods not colored, papery texture, curved, flat, length 9.00, width 1.03 cm. Seeds per pod, 5.4. Apex abrupt. Spur marginal, curved, length 0.730 cm. Dry seeds not reniform, truncated, length 1.444, width 0.764, thickness 0.535 cm. Thickness 0.699 of width. Seeds flat; 3,320 seeds per 1,000 g. Color plum violet (12, p. 172-4), no eye marking. (Figs. 25-71; 32-7.) Very late, low productivity, susceptible to anthracnose. Not well adapted in north central United States. From Oscar Will Seed Co., Bismarck, N. Dak., under name given. Also received under name of Red Indian.

SELECTION 128

Similar to Hidatsa Red, No. 131. Height 70-75 cm, nodes 17-20. Mature pods, length 8.668, width 0.993 cm. Seeds per pod 5. Spur length 0.911 cm. Dry seeds, length 1.139, width 0.706, thickness 0.481 cm. Thickness 0.681 of width; 4,750 seeds per 1,000 g. (Figs. 25-72.) Very susceptible to anthracnose. Medium late, medium productivity; adapted to north central United States.

SELECTION 147

Plant short trailing, height 75 to 120 cm, nodes 13-17. Leaves and stems green throughout. Leaves medium size, short pointed. Flowers white. Mature pods often splashed red, papery texture, curved, flat, length 10.479, width 1.143 cm. Seeds per pod, 4.6. Apex abrupt. Spur marginal, curved, length 1.136 cm. Dry seeds not reniform; length 1.178, width 0.801, thickness 0.517 cm. Thickness 0.645 of width. 3,055 seeds per 1,000 g. Color of seeds snuff brown (12, p. 303). No eye marking. (Figs. 25-74; 32-8.) Medium early, productive, resistant to anthracnose. Adapted to north central United States.

SELECTION 142

Plant short trailing, height 70-75 cm, nodes 14-15. Leaves and stems green throughout. Leaves medium size, broad, short pointed. Flowers white. Mature pods slightly splashed red, papery texture, curved, flat, length 9.712, width 0.965 cm. Seeds per pod, 5.0. Apex abrupt. Spur marginal, curved. Length 0.849 cm. Dry seeds reniform, length 1.33, width 0.724, thickness 0.496 cm. Thickness 0.683 of width. Seeds flat; 3,165 seeds per 1,000 g. Color dull purple lake (12, p. 170-3, 4), stippled white. No eye marking. (Figs. 24-62; 31-9.) Late, medium productivity, resistant to anthracnose. Most nearly resembles Hansen, the variety in which it occurred.

ZEBRA, No. 126

Plant short trailing, height 60-65 cm, nodes 16-17. Leaves dark green, medium size, short pointed. Stems purple. Flowers, standard magenta (12, p. 182-2), wings violet rose (12, p. 154-2). Mature pods splashed purple, medium texture, curved, flat, length 8.847, width 0.907 cm. Seeds per pod 5. Spur marginal, curved, length 0.817 cm. Dry seeds reniform, truncated, length 1.158, width 0.709, thickness 0.494 cm. Thickness 0.696 of width. Seeds flat; 4,018 seeds per 1,000 g. Color smoke gray (12, p. 363), longitudinal black stripes, eye black, no hilum ring marking. (Figs. 24-63; 31-10.) Late, medium productivity, slightly susceptible to anthracnose. Indian booth, South Dakota State fair, and grocery store, Gonvick, Minn.

HANSEN, No. 122

Plant short trailing, height 70-80 cm, nodes 17-18. Leaves and stems green throughout. Leaves medium size, short pointed. Flower color, standard and wings pale reddish lilac (12, p. 131-1). Mature pods not colored, medium texture, curved, flat, length 9.392, width 1.001 cm. Seeds per pod 5.4. Apex abrupt. Spur marginal, curved, length 0.854 cm. Dry seeds, length 1.141, width 0.718, thickness 0.484 cm. Thickness 0.674 of width. Seeds flat; 4,296 seeds per 1,000 g. Color background stone (12, p. 312) tinged lilac mauve (12, p. 196), striped with longitudinal violet (vinous mauve, 12, p. 184) bands. (Figs. 24-64; 32-1.) Late, productive, susceptible to anthracnose. Adapted to the north central part of the United States. Described by Freeman. From the Arizona Agriculture Experiment Station, Tucson, Ariz.

SELECTION 148

Plant short trailing, decumbent, height 70-75 cm, nodes 17-18. Leaves dark green; stems with considerable purple color. Flower color, standard magenta (12, p. 182-2), wings violet rose (12, p. 154-2). Mature pods splashed purple, medium texture, curved, length 9.582, width 0.973 cm. Apex abrupt. Spur marginal, curved, length 0.927 cm. Dry seeds subreniform, truncated, length 1.251, width 0.734, thickness 0.502 cm. Thickness 0.682 of width. Seeds flat, 3,929 seeds per 1,000 g. Color pale yellowish flesh (12, p. 68-2), mottled black. No eye markings. (Figs. 24-65; 32-2.) Late, medium productivity. Susceptible to anthracnose. Selected from a lot of beans secured from the Sisseton Indian Reservation, S. Dak.

GARAYPATA, No. 127

Plant short trailing, decumbent habit, height 60-65 cm, nodes 16-17. Leaves and stems green throughout. Flowers white. Mature pods splashed red, medium texture, curved, length 9.144, width 0.941 cm. Seeds per pod 4.9. Apex abrupt. Spur not marginal, curved, length 0.942. Dry seeds subreniform; length 1.205, width 0.724, thickness 0.498 cm. Thickness 0.688 of width. 3,930 seeds per 1,000 g. Color salmon flesh (12, p. 138-2), mottled snuff brown (12, p. 303). Eye color raw sienna (12, p. 329-1, 2). (Figs. 24-66; 32-3.) Late, medium productivity, susceptible to anthracnose. Described by Freeman (5). Known commercially as Pinto. From Arizona Agricultural Experiment Station, Tucson, Ariz., and several other locations, including the Dakotas.

MOTTLED RED INDIAN, No. 157

Plant short trailing, height 50-66 cm, nodes 24-26. Leaves and stems green throughout. Leaves dark green, broad, short, pointed, highly pubescent. Flowers white. Mature pods not colored, papery texture, markedly curved, flat, length 9.001, width 1.030 cm. Seeds per pod 4.6. Spur marginal, curved, length 0.730 cm. Dry seeds subreniform; length 1.444, width 0.764, thickness 0.535 cm. Thickness 0.699 of width. Seeds flat; 4,347 seeds per 1,000 g. Color plum violet (12, p. 172-4) and white in irregular pattern. No eye markings. (Fig. 24-67.) Very late, low productivity, susceptible to anthracnose. Not adapted to north central United States. From A. B. Shoemaker, Modesto, Calif. Synonym Spotted Red Indian.

BAYOU CHICO, No. 158

Plant short trailing, height 70-72 cm, nodes 19-20. Leaves and stems green throughout. Leaves small, short pointed. Flowers white. Mature pods not colored, papery in texture, curved, length 9.025, width 0.888 cm. Seeds per pod 5.2. Apex abrupt. Spur marginal, curved, length 0.933 cm. Dry seeds reniform, length 1.083, width 0.674, thickness 0.471 cm. Thickness 0.697 of width. 4,616 seeds per 1,000 g. Color raw sienna (12, p. 329-1, 2). No eye marking. (Figs. 24-68; 32-4.) Medium early, medium productivity, highly susceptible to anthracnose. From Arizona Agricultural Experiment Station, Tucson, Ariz., and Germaine Seed Co., Los Angeles, Calif.

BAYOU GRANDE, No. 159

Plant short trailing, height 45-50 cm, nodes 10-11. Leaves and stems green throughout. Leaves large, highly pubescent, blunt pointed. Flowers, standard lilac (12, p. 176-3, 4), wings lilac (12, p. 176-1, 2). Mature pods often splashed red, wrinkled surface, curved, length 11.40, width 0.960 cm. Seeds per pod 4.9. Apex abrupt. Spur marginal, straight, length 0.806 cm. Dry seeds reniform, length 1.323, width 0.804, thickness 0.768 cm. Thickness 0.768 of width. 2,272 seeds per 1,000 g. Color pale pink (12, p. 135-2), eye Roman ocher (12, p. 327), with snuff brown (12, p. 303) hilum ring. Seed color changes to yellow and brown with age or exposure. (Figs. 24-69; 32-5.) Very late, low productivity, susceptible to anthracnose. Not adapted to north central United States. From Germaine Seed Co., Los Angeles, Calif.

PINK, No. 160

Plant short trailing, height 45-50 cm, nodes 22. Leaves and stems green throughout. Leaves large. Flowers white. Mature pods not colored, curved, flat, length 8.862, width 1.060 cm. Seeds per pod 5.2. Apex abrupt. Spur marginal, curved, length 0.804 cm. Dry seeds reniform; length 1.222, width 0.746, thickness 0.550 cm. Thickness 0.737 of width; 4,854 seeds per 1,000 g. Color hydrangea pink (12, p. 132). Not distinctly eyed. (Fig. 25-79.) Very late, susceptible to anthracnose and bean rust. Not adapted to north central United States. From Germaine Seed Co., Los Angeles, Calif.

WILD GOOSE, No. 121

Plant short trailing, height 60-65 cm, nodes 16-17. Leaves dark green, medium size, blunt pointed. Stems with considerable purple color. Flowers, standard magenta (12, p. 182-2), wings reddish violet (12 p. 180-1). Mature pods splashed purple, medium texture, straight, round, length 7.958, width 0.977 cm. Seeds per pod 5.4. Apex abrupt. Spur marginal, curved, length 0.780 cm. Dry seeds not reniform, truncated, length 0.874, width 0.661, thickness 0.502 cm. Thickness 0.759 of width. 5,090 seeds per 1,000 g. Color flesh (12, p. 67-1) mottled sepia (12, p. 300), not distinctly eyed. (Figs. 24-70; 32-6.) Late, productive, resistant to anthracnose and blight. Selected out of Garaypata. Seed obtained as a mixture in Garaypata and later without admixture under the name given.

IMPROVED YELLOW EYE, No. 120

Plants short trailing, height 75-80 cm, nodes 11-12. Mature pods curved, length 9.764, width 1.028 cm. Seeds per pod 4.8. Spur not marginal, curved, length 1.021 cm. Dry seeds, length 1.133, width 0.737, thickness 0.634 cm. Thickness 0.860 of width. Seeds round; 2,572 seeds per 1,000 g. Color white with yellow ocher (12, p. 326-4), pattern around hilum covering approximately one-third of entire area. Eye snuff brown (12, p. 303) with yellow ocher hilum ring. (Figs. 25-75; 32-9.)

RUBY HORTICULTURAL BUSH, No. 129

Plant short trailing, similar to Vineless Marrow. Height 75-80 cm, nodes 11 to 12. Leaves and stems green throughout. Leaves medium size, long pointed. Flowers, standard lilac (12, p. 176-3, 4), wings lilac (12, p. 176-1, 2). Mature pods splashed red, coarse, slightly curved, length 10.217, width 1.070 cm. Seeds per pod 3.8. Apex elongated. Spur marginal, curved, length 1.617

cm. Dry seeds oval, length 1.203, width 0.786, thickness 0.642 cm. Thickness 0.816 of width. 2,281 seeds per 1,000 g. Color flesh (12, p. 139-1) with large irregular splashes of garnet brown (12, p. 164-4), eye Roman ochre (12, p. 327). No distinct hilum ring. (Figs. 25-76; 32-10.) Medium early, productive, susceptible to anthracnose, resistant to blight. From Northrup, King & Co., Minneapolis, Minn.

DISCUSSION

In Table 5 are listed the 43 named varieties and 22 selections of field beans that have been distinguished and classified. Of these, 20 named varieties and 10 selections have white seeds and 23 named varieties and 13 selections have colored seeds.

From the large collection grown it was possible to identify practically all of the field varieties of common beans previously classified and described. Distinct varieties not previously classified and described have been given the names under which they occurred most frequently. Great Northern and Arikara Yellow are the most important of these and Zebra another, probably somewhat less important. One new variety which has several characteristics that may make it valuable in some of the field-bean-growing districts was selected out of the variety Garaypata. It was later obtained in pure form under the name Wild Goose.

In making up the key to the varieties and selections, morphological characters that are stable under varying conditions were employed as far as possible. Therefore the key should be useful in identifying varieties within narrow limits under widely different environments.

Yield data have been secured for practically all the named varieties and for many of the selections. These data have not been used in the classification but serve to indicate the value of the varieties and selections under the environmental conditions prevailing during the seasons in which they were grown.

TABLE 5.—Index to varieties of field beans

Variety name	Minnesota variety or selection No.	Description page	Illustrations of —	
			Seeds	Pods
			Figure	Figure
Arikara Yellow	117	45	24-60	31- 7
Bayou Chico	158	47	24-68	32- 4
Bayou Grande	159	42	24-69	32- 5
Black Turtle Soup	118	46	24-61	31- 8
Blue Pod	102	42	24-44	29-9, 10
Brown Norwegian	133	43	24-49	30- 5
Selection	140	43	24-48	30- 4
Do.	150	43	24-50	30- 6
Do.	141	43	24-51	30- 7
Do.	161	43	24-52	30- 8
Brown Swedish	144	44	24-55	31- 2
Selection	135	44	24-56	31- 3
Do.	145	44	24-57	31- 4
Burlingame	80	40	23-26	27- 10
Selection	154	40	23-28	28- 2
California Wonder	91	40	23-24	27- 8
China Red Eye	151	44	24-58	31- 5
Dark Red Kidney	149	42		
Emperor of Russia	132	43		
Eureka	130	44	24-54	31- 1
French Dwarf Kidney	115	29	23- 7	26- 4
French Dwarf Rice	101		24-41	29- 6
French White	98	40	23-32	28- 6
Garaypata	127	46	24-66	32- 3
Great Northern	78	30	23-12	27- 1
Selection	97	32	23-13	27- 2
Do.	85	32	23-16	27- 3
Hansen	122	46	24-64	32- 1
Selection	142	45	24-62	31- 9
Do.	148	46	24-65	32- 2
Haricot	103	29	23-10	26- 7
Selection	162	29	23-11	26- 8
Hidatsa Red	131	45	25-71	32- 7
Selection	128	45	25-72	
Do.	147	45	25-74	32- 8
Improved Goddard	139	43	24-47	30- 3
Improved Yellow Eye	120	47	25-75	32- 9
Lady Washington	82	32	23-18	27- 4
Selection	106	32	23-19	27- 5

TABLE 5.—Index to varieties of field beans—Continued

Variety name	Minne- sota variety or selec- tion No.	Descrip- tion page	Illustrations of—	
			Seeds	Pods
			Figure	Figure
Long Yellow.....	124	42	24-46	30- 2
Mottled Red Indian.....	157	46	24-67	
Navy Pea.....	94	41	24-36	29- 1
Selection.....	111	42	24-43	29- 8
Do.....	112	42	24-42	29- 7
Old-Fashioned Yellow Eye.....	125	44	24-59	31- 6
Pearce Improved.....	70	46	23-25	27- 9
Pilot.....	83	32	23-22	
Pink.....	160	47	25-79	
Red Kidney.....	116	42	24-45	30- 1
Selection.....	123	42		
Robust.....	76	41	24-40	29- 5
Ruby Horticultural Bush.....	129	47	25-76	32- 10
Selection.....	156	43	24-53	30- 9
Do.....	90	29	23- 9	26- 6
Snowflake.....	75	41	24-37	29- 2
Selection.....	152	41	24-38	29- 3
Vineless Marrow.....	79	41	23-35	28- 9
White Kidney.....	104	29	23- 8	26- 5
White Marrow.....	87	41	23-53	28- 7
Selection.....	100	41	23-34	28- 8
White Wonder.....	92	40	23-30	28- 5
Selection.....	81	40	23-21	28- 4
Wild Goose.....	121	47	24-70	32- 6
Wolverine (see Snowflake).....	110		24-39	29- 4
Yankee Winter.....	107	32	23-23	27- 7
Zebra.....	126	46	24-63	31- 10

LITERATURE CITED

- (1) ANONYMOUS.
1920. DWARF FRENCH BEANS AT WISLEY, 1919. *Jour. Roy. Hort. Soc.* 45: 316-333.
- (2) BAILEY, L. H.
1914. BEAN. In his *Standard Cyclopaedia of Horticulture*, v. 1, p. 458-463, illus. New York and London.
- (3) BURR, F., JR.
1865. THE FIELD AND GARDEN VEGETABLES OF AMERICA: CONTAINING FULL DESCRIPTIONS OF NEARLY ELEVEN HUNDRED SPECIES AND VARIETIES; WITH DIRECTIONS FOR PROPAGATION, CULTURE, AND USE. . . . 667 p., illus. Boston.
- (4) EMERSON, R. A.
1916. A GENETIC STUDY OF PLANT HEIGHT IN *PHASEOLUS VULGARIS*. *Nebr. Agr. Expt. Sta. Research Bul.* 7, 73 p., illus.
- (5) FREEMAN, G. F., JAFFA, A., and DE ONG, E. R.
1912. SOUTHWESTERN BEANS AND TEPARIES. *Ariz. Agr. Expt. Sta. Bul.* 68, p. [573]-619, illus.
- (6) HENDRY, G. W.
1918. BEAN CULTURE IN CALIFORNIA. *Calif. Agr. Expt. Sta. Bul.* 294, p. [287]-347, illus.
- (7) IRISH, H. C.
1901. GARDEN BEANS CULTIVATED AS ESCULENTS. *Missouri Bot. Gard. Rpt.* 12: 81-165, illus.
- (8) JARVIS, C. D.
1908. AMERICAN VARIETIES OF BEANS. *N. Y. Cornell Agr. Expt. Sta. Bul.* 260, p. 149-255, illus.
- (9) LEACH, J. G.
1923. THE PARASITISM OF *COLLETOTRICHUM LINDEMUTHIANUM*. *Minn. Agr. Expt. Sta. Tech. Bul.* 14, 41 p., illus.
- (10) MARTENS, G. VON.
1869. DIE GARTENBOHNEN. IHRE VERBREITUNG, CULTUR UND BENÜTZUNG. Ausgabe 2, vermehrte, 105 p., illus. Ravensburg.

-
- (11) SHAW, J. K., and NORTON, J. B.
1918. THE INHERITANCE OF SEED COAT COLOR IN GARDEN BEANS. Mass. Agr. Expt. Sta. Bul. 185, p. [59]-104.
- (12) SOCIÉTÉ FRANÇAISE DES CHYRSANTHÉMISTES, and OBERTHÜR, R., with the principal collaboration of DAUTHENAY, H.
1905. REPERTOIRE DE COULEURS POUR AIDER À LA DÉTERMINATION DES COULEURS DES FLEURS, DES FEUILLAGES ET DES FRUITS. 82 p., illus. [text] Rennes and Paris. [Text and 2 volumes of plates bound separately.]
- (13) TRACY, W. W., JR.
1907. AMERICAN VARIETIES OF GARDEN BEANS. U. S. Dept. Agr., Bur. Plant Indus. Bul. 109, 160 p., illus.
- (14) ZAVITZ, C. A.
1915. FIELD BEANS. Ontario Dept. Agr., Bul. 232, 15 p., illus.

THE INFLUENCE OF CERTAIN BALANCED RATIONS ON THE CHEMICAL AND PHYSICAL PROPERTIES OF MILK FAT¹

By O. R. OVERMAN, *Assistant Chief in Dairy Chemistry*, and O. F. GARRETT, *First Assistant in Dairy Chemistry, Department of Dairy Husbandry, Illinois Agricultural Experiment Station*

INTRODUCTION

Many results are given in the literature dealing with milk fat which show the effects of different feeds for dairy cows upon the milk fat produced. Amberger (1)² found that the feeding of beets caused an appreciable lowering of the iodine number and an increase in the Reichert-Meissl and Polenske numbers. When sucrose was substituted for the beets the changes were similar in direction but not so great. Amberger also found that when malt germs were fed, the Reichert-Meissl number was reduced from 25.48 to 16.70 and the iodine number was increased from 32.1 to 39.2. Siegfeld (14, 15), Fritzsche (8), and Lubrig, Hepner, and Blau (13), studied the influence of beet leaves and tops on the composition of milk fat. Their results showed that the Reichert-Meissl, Polenske, and saponification numbers of the fat were increased while the iodine number and the index of refraction were lowered. Zaitschek (17) found that cows on a turnip ration produced fat having higher Reichert-Meissl and saponification numbers than when on dry feed, and that potatoes acted similarly to turnips. Boes and Weyland (4) fed a ration of sugar beets exclusively. The fat produced gave the following results: Reichert-Meissl number 28.16, Polenske number 6.16, saponification number 234.2, and iodine number 24.22. Kieferle (10) showed that the feeding of silage gave a considerable increase in the Reichert-Meissl number and a slightly higher Polenske number.

Lindsey, Holland, and Smith (12) state that soybean meal caused no marked difference in milk fat except a slight increase in the unsaturated acids. Soybean oil, however, caused the saponification number to drop from 231.59 to 221.65, and the Reichert-Meissl from 27.05 to 23.27. The iodine number on the other hand increased from 31.90 to 40.25. Cranfield (5) worked with coconut cake and linseed cake and Cranfield and Taylor (6) with linseed cake and hempseed cake. They showed that when cows were removed from poor pasture to well-balanced rations containing these products a considerable rise in the Reichert-Meissl, Kirschner, and Polenske numbers and a fall in the index of refraction occurred. Smith, Wells, and Ewing (16) report that the feeding of cottonseed oil lowered the saponification number and the soluble fatty acids and increased the insoluble fatty acids. Eckles and Palmer (7) showed that cottonseed products in the ration decreased the saponification and Reichert-Meissl numbers

¹ Received for publication July 10, 1931; issued August, 1932. Presented before the Division of Agricultural and Food Chemistry at the eighty-first meeting of the American Chemical Society, Indianapolis, Ind., Mar. 30 to Apr. 3, 1931.

² Reference is made by number (italic), to Literature Cited, p. 57.

and increased the iodine number, while Geisler (9) states that cottonseed feeding gave higher Reichert-Meissl and iodine numbers and a lower Polenske number.

It should be noted that much of the work reported on the effects of feeds upon milk fat has dealt with rations which may be considered abnormal in some respect. For example, rations made up solely of sugar beets, silage, etc., and those to which appreciable amounts of oil, such as cottonseed or soybean oil, have been added are not typical rations for the dairy herd.

The purpose of this investigation was to study the effect on milk fat of different grain mixtures each of which was a normal balanced feed.

GENERAL PLAN OF THE WORK

Three groups of cows were selected, each group producing about 200 pounds of milk daily. These groups were made up as shown in Table 1.

TABLE 1.—*Breed, age, calving, and milk-production data for cows in the various groups*

GROUP 1

Animal No.	Breed	Age on Nov. 1, 1930	Date of last calving	Approximate milk daily on Nov. 1, 1930
		<i>Y. M. D.</i>	<i>1930</i>	<i>Pounds</i>
370.....	Holstein.....	6 6 15	Jan. 18.....	35
355.....	do.....	6 3 27	Aug. 21.....	46
403.....	do.....	4 9 13	May 27.....	45
406.....	do.....	4 4 15	Oct. 18.....	45
428.....	do.....	2 10 15	Mar. 18.....	32

GROUP 2

423.....	Holstein.....	3 5 15	Oct. 23.....	45
431.....	do.....	2 11 18	Apr. 5.....	28
432.....	do.....	2 9 28	Apr. 10.....	25
437.....	do.....	2 9 25	June 8.....	37
438.....	do.....	2 9 7	June 14.....	30
440.....	do.....	2 7 28	July 24.....	33
442.....	do.....	2 0 13	July 13.....	18

GROUP 3

395.....	Brown Swiss.....	8 1 15	May 5.....	34
408.....	Jersey.....	3 11 26	Apr. 2.....	20
412.....	Guernsey.....	6 10 7	Aug. 1.....	25
416.....	Ayrshire.....	3 5 27	Aug. 14.....	28
436.....	Jersey.....	2 9 17	Apr. 29.....	18
439.....	Brown Swiss.....	2 11 28	June 15.....	22
443 ^a	Guernsey.....	2 5 21	Sept. 18.....	15
444 ^a	Ayrshire.....	2 6 23	Oct. 18.....	25

^a Nos. 443 and 444 were dropped from the experiment Dec. 4, 1930, because of the development of a bad flavor in the milk.

Special grain mixtures were made up so that about 50 per cent of the total protein of the mixture was supplied by a high protein feed. Otherwise, each mixture was the same except for a small difference in the percentage of corn. The three special mixtures used were as follows:

Special herd mixture A

	Per cent		Per cent
Cottonseed meal, 43 per cent.....	19	Molasses.....	7.5
Corn, ground.....	21	Bone meal.....	1
Barley, ground.....	15	Salt.....	1.5
Oats, ground.....	20		
Wheat, ground.....	15		100.0

Special herd mixture B

	Per cent		Per cent
Linseed meal.....	24	Molasses.....	7.5
Corn, ground.....	16	Bone meal.....	1
Barley, ground.....	15	Salt.....	1.5
Oats, ground.....	20		
Wheat, ground.....	15		100.0

Special herd mixture C

	Per cent		Per cent
Soybeans (seed), ground.....	23	Molasses.....	7.5
Corn, ground.....	17	Bone meal.....	1
Barley, ground.....	15	Salt.....	1.5
Oats, ground.....	20		
Wheat, ground.....	15		100.0

These mixtures were prepared by a commercial feed manufacturer according to formulas furnished to him. The percentages of total protein were calculated to be: A, 16.65; B, 16.16; and C, 16.38.

The formula for the regular herd mixture is:

	Per cent		Per cent
Barley.....	20	Soybean meal.....	5
Corn.....	7.5	Wheat.....	5
Corn gluten feed.....	15	Wheat bran.....	15
Cottonseed meal.....	5	Bone meal.....	1
Molasses.....	10	Salt.....	1.5
Oats.....	15		

This mixture contained a total protein content of 16.04 per cent.

The grain mixtures were fed in the approximate proportion of 1 pound of the mixture for each 3 pounds of milk produced. Corn silage and good quality alfalfa hay were fed liberally throughout the experiment. There was no pasture or feed in the yard.

The experimental feeding was arranged as shown in Table 2.

TABLE 2.—Arrangement of experimental periods and grain mixtures fed

Period	Date	Grain mixtures fed *
Preliminary.....	Nov. 1-7, 1930.....	Regular herd mixture.
Transition.....	Nov. 8-14, 1930.....	Gradual change to special mixtures.
First.....	Nov. 15-Dec. 12, 1930.....	Mixtures A, B, and C.
Transition.....	Dec. 13-19, 1930.....	Mixtures A, B, and C gradually interchanged.
Second.....	Dec. 20, 1930-Jan. 9, 1931.....	Mixtures A, B, and C.
Transition.....	Jan. 10-16, 1931.....	Mixtures A, B, and C gradually interchanged.
Third.....	Jan. 17-30, 1931.....	Mixtures A, B, and C.
Transition.....	Jan. 31-Feb. 12, 1931.....	Gradual change to regular herd mixture
Fourth.....	Feb. 12, 1931, to end of experiment.	Regular herd mixture.

* Special mixtures A, B, and C were fed to the three groups of cows as follows:

	First period	Second period	Third period
Group 1.....	A	C	B
Group 2.....	C	B	A
Group 3.....	B	A	C

The milk produced by each group of cows was kept separate on certain days during each period of the experiment. Each lot of milk was separated with a centrifugal separator, the cream was churned, and the butter was subjected to laboratory examination.

LABORATORY PROCEDURE

Each lot of butter was melted on a warm water bath and after the water had separated by gravity the fat was decanted and filtered. The determinations reported in this paper are: (1) Index of refraction (a Zeiss butyro-refractometer was used); (2) iodine absorption number (Hanus method); (3) saponification number (Koettstorfer number); (4) soluble acids; (5) insoluble acids (Hegner number); (6) Soluble volatile acids (Reichert-Meissl number); (7) insoluble volatile acids (Polenske number); (8) Jensen-Kirschner number (determined by the method described by Leach (*11, p. 501*)); (9) free fatty acids; and (10) mean molecular weight.³

All determinations except where otherwise noted were made by official methods of analysis of the Association of Official Agricultural Chemists (2).

TABLE 3.—Results of the examination of the milk fats produced by the three groups of cows on various rations

Date	Period	Ration	Refractive index	Iodine number	Saponification number	Soluble acids	Insoluble acids (Hegner number)	Reichert-Meissl number	Polenske number	Jensen-Kirschner number	Free fatty acids	Mean molecular weight
1930												
Nov. 7.	Preliminary	Regular	1.4594	33.38	234.35	5.04	79.48	30.74	2.70	25.48	0.40	239.4
Nov. 15	Transition	Regular to A	1.4592	31.06	234.91	6.77	87.80	29.00	2.88	24.06	37	240.0
Nov. 24	First	A	1.4592	31.02	235.32	4.60	83.06	30.54	3.00	26.29	54	238.2
Nov. 29	do	A	1.4591	32.09	236.62	4.32	81.03	31.58	2.92	27.08	42	237.1
Dec. 7	do	A	1.4588	30.30	236.84	3.68	77.88	26.90	3.02	21.70	1.47	236.9
Dec. 11	do	A	1.4592	32.70	234.27	4.14	75.75	31.44	2.82	25.92	59	222.6
Dec. 18	Transition	A to C	1.4595	33.60	235.70	5.20	77.22	31.26	2.22	26.33	59	238.0
Dec. 26	Second	C	1.4593	33.08	236.14	4.72	81.65	31.36	2.93	29.08	66	237.6
1931												
Jan. 2	do	C	1.4601	38.57	234.26	4.89	86.88	30.94	1.79	26.66	1.01	239.5
Jan. 7	do	C	1.4600	36.46	229.94	4.08	83.12	30.40	2.42	24.78	63	244.0
Jan. 16	Transition	C to B	1.4597	35.28	235.90	4.94	78.69	30.61	2.67	26.26	1.07	237.8
Jan. 25	Third	B	1.4600	36.40	236.24	3.16	86.00	25.96	2.26	20.90	1.60	237.5
Feb. 2	do	B	1.4597	34.28	231.16	3.42	88.41	28.18	2.69	24.42	1.01	242.7
Feb. 19	Transition	B to regular	1.4594	32.84	237.01	3.72	84.61	31.02	2.68	24.56	1.02	236.7
Feb. 25	Fourth	Regular	1.4594	32.41	236.75	4.51	86.34	31.22	2.83	27.05	1.12	237.0
Mar. 3	do	do	1.4590	31.20	236.49	4.28	82.80	30.82	2.88	26.34	1.12	237.2

GROUP 2

1930												
Nov. 7.	Preliminary	Regular	1.4598	34.96	233.84	5.62	80.78	32.89	2.23	24.88	0.35	239.3
Nov. 15	Transition	Regular to C	1.4599	35.52	231.84	5.22	84.04	31.38	2.18	26.99	38	242.0
Nov. 24	First	C	1.4599	33.12	236.53	4.91	82.44	30.06	2.19	26.66	62	237.2
Nov. 29	do	C	1.4599	36.66	232.39	4.78	81.97	30.77	2.23	26.18	32	241.4
Dec. 7	do	C	1.4598	36.58	234.06	3.74	80.50	30.79	2.10	26.88	61	238.7
Dec. 11	do	C	1.4601	37.46	229.70	3.88	74.78	30.80	2.28	28.10	63	227.0
Dec. 18	Transition	C to B	1.4598	35.94	233.20	4.73	79.33	31.36	2.13	25.94	44	240.6
Dec. 26	Second	B	1.4597	35.52	238.94	4.66	83.86	31.44	2.83	27.55	50	237.8

TABLE 3.—Results of the examination of the milk fats produced by the three groups of cows on various rations—Continued

GROUP 2—Continued

Date	Period	Ration	Refractive index	Iodine number	Saponification number	Soluble acids	Insoluble acids (Hehner number)	Reichert-Meissl number	Polenke number	Jensen-Kirschner number	Free fatty acids	Mean molecular weight
1931												
Jan. 2	do	B	1.4597	35.21	234.38	4.69	82.95	29.78	2.06	25.72	.59	239.4
Jan. 7	do	B	1.4598	35.77	231.24	4.24	81.49	29.84	2.54	24.14	.64	242.6
Jan. 16	Transition	B to A	1.4598	34.04	234.29	4.87	79.62	30.63	3.10	25.18	1.01	239.6
Jan. 25	Third	A	1.4602	36.15	230.70	4.01	84.23	27.01	2.10	21.90	1.48	243.2
Feb. 2	do	A	1.4598	33.46	231.18	3.87	87.33	29.98	2.46	25.52	.94	242.7
Feb. 19	Transition	A to regular	1.4594	32.84	230.84	4.46	86.10	31.42	2.52	25.82	.94	236.9
Feb. 25	Fourth	Regular	1.4594	32.40	235.56	4.29	85.92	30.00	2.80	25.77	1.13	238.2
Mar. 3	do	do	1.4592	32.64	235.10	4.33	84.76	31.50	2.78	27.70	.95	238.6

GROUP 3

1930												
Nov. 7	Preliminary	Regular	1.4591	29.72	237.21	6.85	84.67	28.72	3.16	21.82	0.32	236.5
Nov. 15	Transition	Regular to B	1.4589	32.13	235.36	5.66	82.23	31.96	2.93	23.58	.43	237.7
Nov. 24	First	B	1.4590	31.32	241.05	4.92	83.10	30.60	3.20	24.98	.32	232.8
Nov. 29	do	B	1.4590	30.93	238.26	4.02	80.42	30.30	3.07	27.48	.45	235.5
Dec. 7	do	B	1.4587	32.26	236.58	5.01	82.16	30.62	2.55	24.31	.96	237.1
Dec. 11	do	B	1.4588	29.34	236.72	4.66	80.08	28.78	2.94	24.70	.68	237.1
Dec. 18	Transition	B to A	1.4591	30.04	236.46	4.02	83.87	28.60	3.28	24.64	.48	237.2
Dec. 26	Second	A	1.4591	30.89	236.96	4.38	81.43	30.32	3.43	26.56	.82	236.8
1931												
Jan. 2	do	A	1.4591	31.54	235.06	4.29	84.02	29.50	2.37	25.42	.78	238.7
Jan. 7	do	A	1.4591	29.72	233.94	4.16	81.83	29.79	3.02	23.82	.40	239.8
Jan. 16	Transition	A to C	1.4595	34.50	234.47	4.68	82.28	30.32	2.55	25.52	.04	239.3
Jan. 25	Third	C	1.4598	34.48	233.82	4.53	83.74	29.50	2.52	22.90	1.59	239.9
Feb. 2	do	C	1.4595	34.11	231.60	3.85	87.32	31.04	2.32	27.00	.40	242.2
Feb. 19	Transition	C to regular	1.4591	31.00	238.02	4.16	84.36	31.49	2.68	24.48	1.32	235.7
Feb. 25	Fourth	Regular	1.4592	32.18	237.33	4.94	85.18	32.50	2.88	27.26	.60	236.4
Mar. 3	do	do	1.4592	31.22	235.38	4.37	84.04	30.96	2.64	25.86	.96	238.4

RESULTS

The results of the determinations on each sample are given in Table 3. In each case the data are placed in the same line with the type of ration which was being fed when the sample was taken.

Table 4 gives the average values for the milk fats produced by each of the three groups of cows that were being fed each of the four grain mixtures and also the average values for all the samples taken from all groups of cows while on each of the four grain mixtures.

A study of the tabulated data shows no very great differences in the values found for the individual samples of fat produced during the feeding of the different grain mixtures. In many cases the fluctuations between individual samples produced while one mixture was being fed were greater than those between other samples produced during the feeding of different mixtures. However, an inspection of the average values shows certain rather definite tendencies. The averages for

¹ The mean molecular weight of the fat was computed by the use of the formula: Mean molecular weight = $\frac{W \times 6000}{g}$, in which W = weight of fat used and g = ml of N/2 KOH used in the saponification, by Bolton (5, p. 36).

all samples (Table 4) show that the fat produced while the cows were on ration C containing ground soybeans has a higher index of refraction and iodine absorption number and a slightly higher mean molecular weight, but a lower saponification number and a lower Polenske number, than the fats produced during the feeding of the three other grain mixtures. These tendencies are in most cases apparent in the averages for the three groups of cows but are not so distinctly evident.

TABLE 4.—Average values for the milk fats produced by the three groups of cows, and by all the cows, on various rations

GROUP 1										
Ration	Refractive Index	Iodine number	Saponification number	Soluble acids	Insoluble acids (Hehner number)	Reichert-Meisssl number	Polenske number	Jensen-Kirschner number	Free fatty acids	Mean molecular weight
Regular	1.4593	32.33	235.86	4.61	82.87	30.93	2.80	26.29	1.21	237.9
A	1.4591	31.28	235.76	4.18	79.43	30.14	2.95	25.25	.76	233.7
B	1.45985	35.34	233.70	3.29	87.50	27.07	2.42	22.66	1.30	240.1
C	1.4598	36.04	233.45	4.56	82.88	30.90	2.38	26.81	.77	240.4
GROUP 2										
Regular	1.4595	33.33	234.83	4.75	83.82	31.45	2.60	26.12	1.14	238.7
A	1.4900	34.80	230.94	3.94	85.78	28.50	2.28	23.71	1.21	243.0
B	1.4597	35.50	233.85	4.53	82.77	30.35	2.48	25.00	.58	239.9
C	1.4599	37.18	233.17	4.33	79.89	29.98	2.20	25.42	.54	236.3
GROUP 3										
Regular	1.4592	31.04	236.04	5.39	84.63	30.73	2.89	24.98	0.63	237.1
A	1.4591	30.72	235.32	4.27	82.43	29.87	2.94	25.27	.67	238.4
B	1.4589	30.96	238.15	4.65	81.59	30.07	2.94	25.37	.60	235.6
C	1.4590	34.30	232.71	4.19	85.53	30.27	2.42	24.95	1.00	241.0
ALL COWS										
Regular	1.4593	32.23	235.78	4.91	83.77	31.04	2.77	25.80	0.99	237.9
A	1.4593	31.98	234.54	4.16	81.84	29.63	2.80	24.91	.83	237.3
B	1.4593	33.45	235.72	4.31	83.30	29.50	2.67	24.94	.75	238.1
C	1.4598	36.16	233.16	4.38	82.43	30.33	2.31	25.79	.72	238.7

SUMMARY AND CONCLUSIONS

The milk fat produced by three groups of cows during the feeding of different grain mixtures has been studied. The conclusions that may be drawn from this work are:

Milk fats produced during the feeding of grain mixtures so prepared that approximately half the protein content is supplied by (1) cottonseed meal, (2) linseed meal, and (3) ground soybeans differ to some extent in some of their physical and chemical characteristics.

Milk fats produced during the feeding of a grain mixture so prepared that half its protein is from ground soybeans, show on an average a higher index of refraction and iodine number, slightly higher mean molecular weight, and lower saponification and Polenske num-

bers than those produced during the feeding of the other grain mixtures used in this work.

The differences between the fats produced by cows fed the grain mixtures used in this investigation do not appear to be large enough to be of other than scientific interest.

LITERATURE CITED

- (1) AMBERGER, C.
1907. DER EINFLUSS DER FÜTTERUNG AUF DIE ZUSAMMENSETZUNG DES BUTTERFETTES. *Ztschr. Untersuch. Nahr. u. Genussmtl.* 13: 614-621.
- (2) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1925. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. REVISED TO JULY 1, 1924. Ed. 2, 535 p., illus. Washington, D. C.
- (3) BOLTON, E. R.
1928. OILS, FATS, AND FATTY FOODS, THEIR PRACTICAL EXAMINATION; A HANDBOOK FOR THE USE OF ANALYTICAL AND TECHNICAL CHEMISTS AND MANUFACTURERS . . . 416 p., illus. Philadelphia.
- (4) BOES, J., and WEYLAND, H.
1915. ZUR KENNTNIS DES EINFLUSSES DER ZUCKERRÜBENFÜTTERUNG AUF DIE ZUSAMMENSETZUNG DES MILCHFETTES. *Ztschr. Untersuch. Nahr. u. Genussmtl.* 29: 473-475.
- (5) CRANFIELD, H. T.
1911. THE EFFECT OF FEEDING WITH COCOANUT CAKE AND LINSEED CAKE ON THE COMPOSITION OF BUTTER-FAT. *Analyst* 36: [445]-449, illus.
- (6) ——— and TAYLOR, M. G. D.
1915. THE EFFECT OF FEEDING ON THE COMPOSITION OF MILK AND BUTTER: LINSEED CAKE AND HEMPSEED CAKE. *Analyst* 40: 433-439, illus.
- (7) ECKLES, C. H., and PALMER, L. S.
1916. EFFECTS OF FEEDING COTTONSEED PRODUCTS ON THE COMPOSITION AND PROPERTIES OF BUTTER. INFLUENCE OF THE CHARACTER OF THE RATION AND QUANTITY OF COTTONSEED PRODUCTS. *Missouri Agr. Expt. Sta. Research Bul.* 27, 44 p., illus.
- (8) FRITZSCHE, M.
1909. BEITRAG ZUR KENNTNIS DER ZUSAMMENSETZUNG DES BUTTERFETTES BEI RÜBENBLATTFÜTTERUNG. *Ztschr. Untersuch. Nahr. u. Genussmtl.* 17: 533-536.
- (9) GEISLER, J. F.
1926. THE EFFECT OF COTTONSEED FEEDING ON BUTTERFAT. *Jour. Oil & Fat Indus.* 3: 115-118.
- (10) KIEFERLE, F.
1923. DER EINFLUSS DER VERFÜTTERUNG VON GARFÜTTER AUF DIE ZUSAMMENSETZUNG DES MILCHFETTES. *Milchw. Forsch.* 1: [2]-14.
- (11) LEACH, A. E.
1920. FOOD INSPECTION AND ANALYSIS, FOR THE USE OF PUBLIC ANALYSTS, HEALTH OFFICERS, SANITARY CHEMISTS, AND FOOD ECONOMISTS. Rev. and enl. by A. L. Winton. Ed. 4, 1090 p., illus. New York.
- (12) LINDSEY, J. B., HOLLAND, E. B., and SMITH, P. H.
1909. EFFECT OF SOYBEAN OIL UPON THE COMPOSITION OF MILK AND BUTTERFAT, AND UPON THE CONSISTENCY OR BODY OF BUTTER. *Mass. Agr. Expt. Sta. Rpt.* 21 (pt. 2): 66-110.
- (13) LÜHRIG, H., HEPNER, A., and BLAU, G.
1909. UEBER DIE BEEINFLUSSUNG DER ZUSAMMENSETZUNG DES BUTTERFETTES DURCH RÜBEBLATTFÜTTERUNG. *Pharm. Zentralhalle* 50: [275]-282.
- (14) SIEGFELD, M.
1907. DER EINFLUSS DER VERFÜTTERUNG VON RÜBENBLÄTTERN UND RÜBENKÖPFEN AUF DIE ZUSAMMENSETZUNG DES BUTTERFETTES. *Ztschr. Untersuch. Nahr. u. Genussmtl.* 13: [513]-524.
- (15) ———
1909. DIE ZUSAMMENSETZUNG DES BUTTERFETTES BEI RÜBENBLATTFÜTTERUNG. *Ztschr. Untersuch. Nahr. u. Genussmtl.* 17: [177]-181.

-
- (16) SMITH, F. H., WELLS, C. A., and EWING, P. V.
1916. THE CHANGES IN COMPOSITION OF BUTTERFAT PRODUCED BY FEEDING
COTTONSEED OIL. Ga. Agr. Expt. Sta. Bul. 122, p. [95]-111.
- (17) ZAITSCHEK, A.
1911. ÜBER DEN EINFLUSS DER FUTTERMITTEL AUF DIE ZUSAMMENSETZUNG
DES MILCHFETTES. Landw. Vers. Sta. 74: [250]-262.

THE MIGRATION OF *BACILLUS AMYLOVORUS* IN THE TISSUE OF THE QUINCE¹

By HERBERT A. WAHL²

Instructor in Botany, Pennsylvania Agricultural Experiment Station

HISTORICAL REVIEW

The fire-blight organism, *Bacillus amylovorus* (Burr.) Trev., is of considerable economic importance because of the disease it produces on three hosts—apple, pear, and quince. A study of the comparative cytological reactions of host and parasite for these three species is of particular biological interest.

Nixon (5, p. 9)³ first described the annual life cycle of *Bacillus amylovorus* in apple tissue. According to this author, the life cycle consists of two stages. The first stage is characterized by the formation of zoogloecae in which the bacteria migrate intercellularly, with the formation of schizogenous cavities "by toxic plasmolysis and crushing." The regions invaded by the bacteria during this stage are described as follows:

* * * the "optimum path" of migration is confined to the intercellular spaces of a zone of cells in the inner cortex * * *. From the "optimum region" the invasion of adjacent, less favorable tissues, may follow * * *. The last tissues to be invaded are the cambium, the xylem and pith, and these are rarely seriously attacked.

In the second or "pseudo-fructification" stage the bacteria become smaller and invade the cells by dissolving the cell wall, thus forming lysigenous cavities. The second stage culminates in the formation of cysts within the cells, in which condition the organism passes the winter.

Haber (2, p. 10) found that under artificial conditions young apple leaves may be the portal of infection; that the bacteria migrate in the form of zoogloecae in the intercellular spaces of the vein parenchyma and spongy mesophyll of the leaf, and that death of the tissues is caused by "plasmolysis of the protoplast, the collapse of cell walls, and the separation of contiguous cell walls to form schizogenous cavities."

The results of Gibbons's⁴ investigation concerning the invasion of the organism and its effect on the host tissue in pear paralleled Nixon's results with apple. Tullis (7) also corroborated Nixon's work as to the general features of the migration of the organism.

Rosen (6), in studying the invasion of the floral structures and petioles of pear and the stems of apple and pear, also reported migration as taking place in the spaces between the cortical cells. The middle lamellae and the cell walls of these cells are broken apart by

Received for publication July 10, 1931; issued August, 1932. Contribution from the Agricultural Experiment Station of the Pennsylvania State College, No. 559, and from the Department of Botany, No. 76.

¹ The writer wishes to express his appreciation for the assistance and helpful suggestions of Dr. E. L. Nixon, under whose direction these investigations were conducted. He is also grateful to Dr. H. W. Thurston and others for valuable suggestions in the preparation of the manuscript.

² Reference is made by number (italic) to Literature Cited, p. 63.

³ GIBBONS, F. P. THE MIGRATION OF *BACILLUS AMYLOVORUS* IN PEAR TISSUE. (Unpublished master's thesis, Pennsylvania State College.)

chemical dissolution with no signs of pressure observable on the cells. This dissolution is noted as occurring ahead of the invading mass of bacteria. The movement of the mass of bacteria in the intercellular spaces is considered "a passive one engendered by the accretion of great numbers of bacteria rather than an active pseudopodlike movement * * *." Rosen reports that all the stem tissues are later invaded and that death of the host is attributable to the formation of cavities by dissolution, particularly in the phloem and cambium, rather than to toxic products secreted by the pathogene.

A review of the literature reveals no reference to any microscopic studies of the invasion of the tissues of the quince (*Cydonia vulgaris* Pers.) by this organism.

METHODS

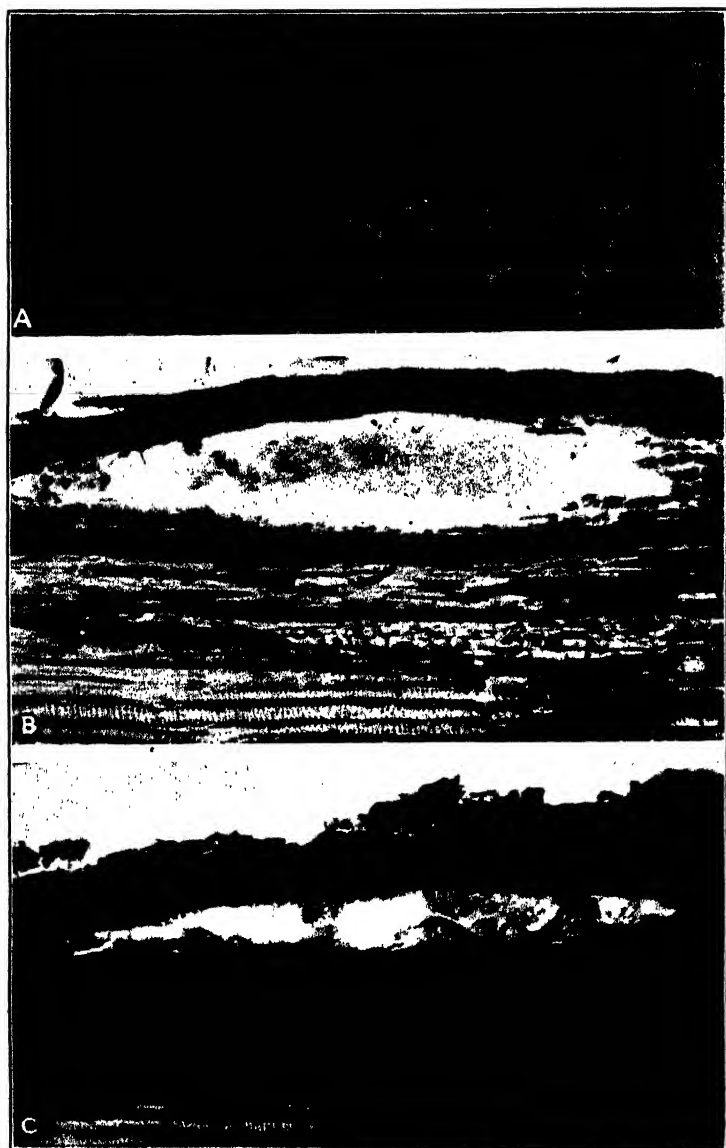
The research presented in this paper was begun at the Pennsylvania State College in the spring of 1925, when a series of inoculations was made on young quince trees growing in the college greenhouse. A pure culture of *Bacillus amylovorus* had been secured previously by isolating the bacteria from blight cankers that had been brought into the laboratory and kept in a moist chamber until the gummy exudate characteristic of this organism appeared. The culture was grown on Lima bean agar and its pathogenicity proved by inoculation of quince trees and reisolation of the organism. Inoculations were made with a sharp laboratory needle introduced at the growing tip of the twig, the puncture being made entirely through the twig. Sections were taken after $\frac{1}{2}$, 1, $1\frac{1}{2}$, 2, 4, 6, 8, 10, 12, 16, 18, 24, and 48 hours, fixed in Flemming's weak solution, and embedded in paraffin by the usual method. In the spring of 1927 a duplicate series of inoculations was made on quince trees growing in the college orchard. The method of inoculating and embedding was the same as that used in the earlier experiments except that sections were taken up to 142 hours after inoculation and were fixed in Flemming's weak, Petrunkevitch's, and alcohol-formalin-acetic fixing fluids. Sections were cut 9μ to 11μ thick and stained with Flemming's triple stain.

OBSERVATIONS

Microscopic examination of the early stages following inoculation showed the bacteria lying along the edge of the puncture, where they had multiplied rapidly in the broken remains of the injured cells. Invasion of the uninjured tissues was found to have taken place in a narrow cylindrical area in the inner cortex extending around the stem about 10 cells inward from the epidermis and several cells outward from the phloem region. In this area the bacteria were found in zoogloal masses invading the intercellular spaces in all directions from the puncture, though more rapidly in a longitudinal than in a tangential direction. Radially the intercellular spaces were occupied only in a layer three to five cells wide. No invasion beyond the puncture was observed one-half hour after inoculation, although at that time large masses of bacteria were present in the puncture. (Pl. 1, A.) After 4 hours the bacteria had reached a distance of about 0.037 mm from the puncture (pl. 1, B), and after 12 hours they had reached a distance of 0.5 mm (pl. 1, C). At this stage none of the cells in the infected area were occupied nor were the cell contents in any way injured, except for a slight plasmolysis.



- A.—Bacteria in the puncture, one-half hour after inoculation. The broken remains of cells, including a xylem vessel, are present. \times about 1,750.
- B.—Zoogloeal mass formed at edge of puncture invading intercellular spaces of inner cortical region, four hours after inoculation. \times about 1,000.
- C.—Tip of zoogloea, 12 hours after inoculation. \times about 1,250.
- D.—Bacteria occupying a space between cortical cells with evidence of slight pressure and splitting of cell walls, 42 hours after inoculation. \times about 1,750.
- E.—The beginning of lysigenous cavity formation by the dissolution of cell wall and protoplasm. The crystal in the cell is not attacked by the bacteria. \times 1,000.



- A.—The formation of a lysigenous cavity by the dissolution of adjacent cortical cells; 110 hours after inoculation at a distance of 2.3 cm from puncture. \times about 185.
- B.—An extremely large lysigenous cavity in the cortex; 66 hours after inoculation adjacent to puncture. All the cortical cells are dead, but there is no noticeable effect on phloem, cambium, xylem, or pith cells. \times about 185.
- C.—Mostly tangential section of cortex showing death to all cells; 142 hours after inoculation adjacent to puncture. \times about 185.

After 42 hours the bacteria had traveled a distance of about 1.5 mm from the point of entrance and had formed numerous, though not large, schizogenous cavities. (Pl. 1, D). Certain cells were split apart at the middle lamella, and the cell walls were slightly invaginated. Also, during this time and in the same region, a few cells were seen to be entirely filled with bacteria. These cells retained their normal outline, while the protoplast was not destroyed but was pushed to one end of the cell, where it became an almost or quite homogeneous mass. Although the complete life cycle of *Bacillus amylovorus* is not being considered here, this condition corresponds closely with the early stages shown by Nixon in apple in which the bacteria multiply rapidly within the lumen of a cell, eventually rounding up to form a cystlike body that is the overwintering form of the organism.

After a time the organism apparently changes its method of invasion and migrates without regard for cells or intercellular spaces. In this stage cell walls and protoplasts alike succumb to the invading horde of bacteria. Cells are entirely broken down, so that no indication of their previous existence remains. The bacteria are now arranged in thin radiating strands in the lysigenous cavities thus formed, their arrangement suggesting that they have utilized the cell wall, nucleus, and cytoplasm for food and have thus formed a strand of bacteria where once there was a strand of cytoplasm or a cell wall. This takes place within 96 hours after inoculation, when the bacteria have traveled a distance of 1 to 1.5 cm from the puncture, and continues until the entire inner cortical region is broken down, the cells shrunken, and their protoplasts entirely disorganized. (Pls. 1, E, and 2, A, B, C.) This is the condition at 142 hours after inoculation, when the invasion has extended longitudinally from the puncture at least 5 cm. Stages later than 142 hours after inoculation have not been studied.

DISCUSSION

The results secured in the present study agree with those of Nixon (5) and Haber (2) in their work with apple and of Gibbons⁴ with pear, thus showing that there is to be found no essential difference in method of migration in any of the three economic hosts of this parasite. These studies also show essential agreement with the work of Hill with *Bacterium tumefaciens* in tomato (3) and *Bact. tabacum* in tobacco (4) and of Beach with *Bact. vignae* in Lima bean (1), so far as the method of migration of the bacterial pathogene is concerned.

The zoogloal mass invaded the tissues at an average rate of 9μ per hour for the first 4 hours, 42μ per hour for the first 12 hours, 36μ per hour for the first 42 hours, 156μ per hour for the first 96 hours, and 352μ per hour for 142 hours. These figures represent only one to several measurements in each case. Although the figures are open to error because of the small number of measurements and because of the difficulty of determining the exact extent of the invasion, a marked difference in rate is shown between the earlier and later stages. Thus, for the 38 hours from the 4 to the 42 hour period after inoculation, the average rate of migration was 38μ per hour. During the next 54 hours the average rate had increased to

⁴ GIBBONS, F. P. Op. cit.

about 250μ per hour, and the average for the next 46 hours was 761μ per hour. Haber found that the zoogloae in apple leaves traveled a distance of 0.5715 mm in 12 hours and had reached a distance of 1.5 mm from the puncture after 24 hours. This is an average rate of 47.6μ per hour for the first 12 hours and 62.5μ per hour for the first 24 hours. Nixon reports a migration of 3 or 4 inches in 24 to 48 hours after inoculation in apple stems. Four inches in 48 hours represents a rate of $2,115\mu$ per hour. It would seem desirable that further work be done with the different hosts under controlled conditions of temperature, humidity, age, and rate of growth of host, etc., to determine, if possible, the factors responsible for such a wide range in the rate of migration.

Nixon found that the region of intercellular migration in apple may extend entirely around the stem and, longitudinally, may involve the entire twig and adjacent limb, and under optimum conditions may extend several feet. In this study the bacteria were found entering the cells of quince within a distance of 1.5 cm from the point of inoculation, and were not found invading the entire circumference of quince stems through the intercellular spaces.

During this intercellular migration some cells in the path of the invading mass of bacteria become separated along the line of the middle lamella. How this separation takes place is still problematical. Two possibilities, however, present themselves as primary factors—either the pressure from the mass of bacteria is great enough to separate the cells or some solvent action of the products of the organism causes the dissolution of the middle layers of the cell wall. Slight evidence of pressure exerted upon the cell is present in the form of indentations in the cell wall. (Pl. 1, D.) Since, however, the cells all through the region of intercellular migration and schizogenous cavity formation are slightly plasmolyzed, it is evident that the cell turgor in these cells is quite low or is, perhaps, entirely lacking. Thus very little pressure would need to be exerted by the bacterial mass to cause invagination of the cell wall. The evidence, then, indicates that only a very slight amount of mechanical pressure is exerted by the invading mass of bacteria. This leads to the conclusion that the cells are split apart by some solvent action of the bacteria.

Many stages in the dissolution and disorganization of cells resulting from bacterial activity were found. Plate 1, E, shows the bacteria at work in the destruction of the wall and contents of a single cell. In Plate 2, A, may be seen an early stage in the formation of a lysigenous cavity by the breaking down of adjacent cells. Advanced stages of these processes are shown in Plate 2, B and C, where extensive cavities have been formed by the total disintegration of several layers of cells in the most susceptible region in the inner cortex. The lysigenous cavity thus formed (pl. 2, A) measures 80μ by 425μ . By examining serial sections this cavity was found to extend in a tangential direction around the stem at least 150μ , each section showing the cavity occupied by a mass of bacteria. The preparations contained many such cavities, each showing the bacteria in good condition. This would seem to contradict the statements of Tullis (?) and Rosen (6) as to the inadequacy of stained paraffin sections for preserving the correct condition and relation of *Bacillus amylovorus* and its host.

There seems to be no reason to doubt that the cell walls and contents are acted upon by growth products of the bacteria and broken

down just as other food materials are broken down by other bacteria. The finer details of the dissolution of the host cells and the exact nature of the method by which the bacteria produce the dissolution are not within the scope of this investigation. Far more important are the reasons for the death of all the tissues, even those not directly attacked by the organism, by the time the organism has been active in the stem for 100 hours. The organism was not found in any tissues of young quince stems except the narrow cortical region previously described. In spite of this, tissues, including phloem, cambium, and pith, are definitely shown to be dead adjacent to the area of cortical invasion. (Pl. 2, C.)

These results are in contrast to those reported by Rosen (6), who states that in apple stems "* * * by far the most serious effects on the infected twig or limb result * * * from invasion of the phloem and cambium. It is the destruction of the latter and not the cortex which results in the death of the twig or limb." Rosen states further (6, p. 64) that "* * * the evidence is indeed substantial for the assumption that diffusible toxic products are not produced by *B. amylovorus*." If Rosen's results are correct, then we are confronted by the fact that the same organism must operate in an entirely different manner to produce the results here recorded for quince. Some tissues of quince stems are killed without being invaded by the organism. The tissues that are invaded are not those directly concerned with either growth or food conduction. The destruction of the tissues invaded is not sufficient to explain the death of the entire twig. Therefore, the evidence is substantial for assuming that diffusible toxic products of *Bacillus amylovorus* are responsible for the death of the stem tissues of quince not invaded by the organism.

SUMMARY

Bacillus amylovorus migrates through the intercellular spaces of the inner cortex of quince in the form of zooglaeae.

During this invasion schizogenous cavities are produced in quince in a manner similar to that previously reported by Nixon in apple.

Intracellular invasion of the cortex, involving formation of lysigenous cavities, occurs within 96 hours after inoculation.

Death of all the stem tissues occurs within 100 hours after intercellular invasion and within 48 hours after intracellular invasion of the adjacent cortex, although during this time the organism is present only in the cortex.

LITERATURE CITED

- (1) BEACH, W. S.
1928. THE RELATION OF BACTERIUM VIGNAE TO THE TISSUES OF LIMA BEAN. Penn. Agr. Expt. Sta. Bul. 226, 15 p., illus.
- (2) HABER, J. M.
1928. THE RELATIONSHIP BETWEEN BACILLUS AMYLOVORUS AND LEAF TISSUES OF THE APPLE. Penn. Agr. Expt. Sta. Bul. 228, 15 p., illus.
- (3) HILL, J. B.
1928. THE MIGRATION OF BACTERIUM TUMEFACIENS IN THE TISSUE OF TOMATO PLANTS. Phytopathology 18: 553-564, illus.
- (4) ———
1929. MIGRATION OF BACTERIUM TABACUM THROUGH THE LEAF TISSUES OF NICOTIANA TABACUM. (Abstract) Phytopathology 19: 97.

-
- (5) NIXON, E. L.
1927. THE MIGRATION OF *BACILLUS AMYLOVORUS* IN APPLE TISSUE AND ITS EFFECT ON THE HOST CELLS. Penn. Agr. Expt. Sta. Bul. 212, 16 p., illus.
- (6) ROSEN, H. R.
1929. THE LIFE HISTORY OF THE FIRE BLIGHT PATHOGEN, *BACILLUS AMYLOVORUS*, AS RELATED TO THE MEANS OF OVERWINTERING AND DISSEMINATION. Ark. Agr. Expt. Sta. Bul. 244, 96 p., illus.
- (7) TULLIS, E. C.
1929. STUDIES ON THE OVERWINTERING AND MODES OF INFECTION OF THE FIRE BLIGHT ORGANISM. Mich. Agr. Expt. Sta. Tech. Bul. 97, 32 p., illus.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 45

WASHINGTON, D. C., JULY 15, 1932

No. 2

SOME PHYSIOLOGICAL STUDIES OF GLOEOSPORIUM PERENNANS AND NEOFABRAEA MALICORTICIS¹

By ERSTON V. MILLER,

Assistant Physiologist, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture²

INTRODUCTION

In certain sections of the Pacific Northwest the control of perennial canker and northwestern anthracnose has become a problem of great economic importance to the apple industry. Perennial canker has been attributed to *Gloeosporium perennans* Zeller and Childs (13),^{3,4} and northwestern anthracnose is caused by *Neofabraea malicortici* (Cordley) Jackson. Both organisms are associated with cankers on the limbs and trunk of the apple tree, and both produce rots on the fruits.

Northwestern anthracnose is known to have been of economic importance since 1891. The causal organism was described in 1900 by Cordley (6) and also by Peck (12, p. 21), Cordley giving it the name of *Gloeosporium malicortici*. In 1913 Jackson (10) described the perfect stage of the fungus, changing the name to *Neofabraea malicortici*.

In 1925 Zeller and Childs (13) reported the occurrence of perennial canker and described as the causal organism *Gloeosporium perennans*. Later in the same year Fisher (7) reported evidence that this disease had been in the Northwest for at least 11 years.

There is great similarity in the cankers and also in the rots produced by the two organisms, and it is generally agreed that the two diseases must have existed side by side for a number of years without any suspicion that two organisms were concerned. It was when the measures recommended for the control of anthracnose were found to be ineffective under Hood River conditions that Zeller and Childs (13) discovered the new species in the cankers. The chief distinctions they made between the two organisms at that time were that the perennial-canker fungus is a wound parasite but perennial in its nature when once established, whereas the anthracnose fungus is able to infect the uninjured bark but does not spread beyond the limits of the first year's growth; that the anthracnose organism has more active diastatic power than the perennial-canker organism and has curved or hooked conidia in contrast to the generally ellipsoid spores

¹ Received for publication Dec. 10, 1931; issued August, 1932.

² The writer is grateful to J. S. Cooley, of the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, and S. M. Zeller, of the Oregon Agricultural Experiment Station, for furnishing cultures of the fungi studied; and to Charles Brooks, of the Division of Horticultural Crops and Diseases, for helpful advice and suggestions.

³ Reference is made by number (italic) to Literature Cited, p. 76.

⁴ Since this paper was prepared, Nellie A. Brown, of the Division of Horticultural Crops and Diseases, has reported (2a) that *Gloeosporium perennans* is frequently absent in otherwise typical cankers, that other fungi as well as bacteria are frequently found, and that under proper conditions typical cankers can be produced by artificial inoculation with these organisms as readily as with *G. perennans*.

of *Gloeosporium perennans*. More recently, however, Childs (3) has reported that the perennial-canker organism is not truly perennial, and Fisher and Reeves (8) have found evidence that it can produce cankers without the aid of previous injuries to the bark, thus reducing the number of well-established distinctions between the two organisms. But in spite of the great similarity of the two diseases, there seems to be no doubt that a new canker organism has appeared in the Northwest, and its control has become one of the outstanding problems in plant pathology.

The present work was undertaken with the hope that further information regarding the physiological responses of the two organisms might throw light on their relationship and also be of value in developing methods of control. Following out this idea, studies have been made of the effect of temperature, acidity, and nutrient salts upon the growth of the organisms.

EXPERIMENTAL PROCEDURE AND RESULTS

CULTURE MEDIA

The two canker organisms from the Northwest were grown on variously modified culture media. The canker organisms comprised authentic cultures of *Neofabraea malicorticis* and *Gloeosporium perennans* received from J. S. Cooley, at Hood River, Oreg., and three strains of each organism obtained from S. M. Zeller, of Corvallis, Oreg. The same methods were used in all the tests. Ten cubic centimeters of the medium were poured into a sterile Petri dish and allowed to solidify. Inoculations were then made in the center of the plate with either spores or mycelial tufts of the organism, and records of growth were taken at regular intervals.

It was found that all of the organisms could be satisfactorily grown on Dox's synthetic agar, so this was selected as the standard medium from which various modifications were made. The following is the standard formula for Dox's agar:

Distilled water.....	cubic centimeters..	1, 000
Magnesium sulphate.....	grams..	0. 5
Dipotassium phosphate.....	do.....	1
Potassium chloride.....	do.....	. 5
Ferrous sulphate.....	do.....	. 01
Sodium nitrate.....	do.....	2
Agar.....	do.....	15

The agar was prepared in three lots: (1) Dox's agar with 0.5 per cent dextrose; (2) as in 1, but lacking potassium chloride; (3) as in 1, but lacking sodium nitrate. When any one of the constituents of Dox's agar is omitted, it may be difficult to obtain a clear solution. To obviate this difficulty monobasic potassium phosphate (KH_2PO_4) was substituted for the dibasic potassium phosphate (K_2HPO_4). In other experiments potash was completely eliminated from the nutrients by substituting monobasic ammonium phosphate for the monobasic potassium phosphate besides omitting the potassium chloride.

The cultures were held at temperatures of 0°, 2.5°, 5°, 10°, 15°, and 20° C. in the refrigerator boxes described by Brooks and Cooley (2).

When the formula for Dox's agar contained either monobasic or dibasic potassium phosphate, the growth of the organisms was not inhibited by the absence of either potassium chloride or sodium nitrate

from the nutrients added to the agar. When monobasic ammonium phosphate was employed, both *Neofabraea malicortici* and *Gloeosporium perennans* were moderately inhibited in their growth at 15° C. by the absence of sodium nitrate or potassium chloride from the nutrients, whereas at 5° and 10° only the lack of potassium chloride had any effect. (Fig. 1.) The rate of inhibition, however, was about as pronounced for one organism as for the other. The slight differences between the two are hardly significant, especially since there was always some variation in the rate of growth of the two organisms.

HYDROGEN-ION CONCENTRATION

A study was made of the responses of the anthracnose and perennial-canker organisms to different hydrogen-ion concentrations. Apple agar was prepared from ripe Delicious apples and the desired pH values were obtained by the addition of N/1 HCl or N/1 NaOH. A set of buffer solutions as designed by Clark and Lubs (5) were prepared for pH values ranging from 1.2 to 11.8. These were made

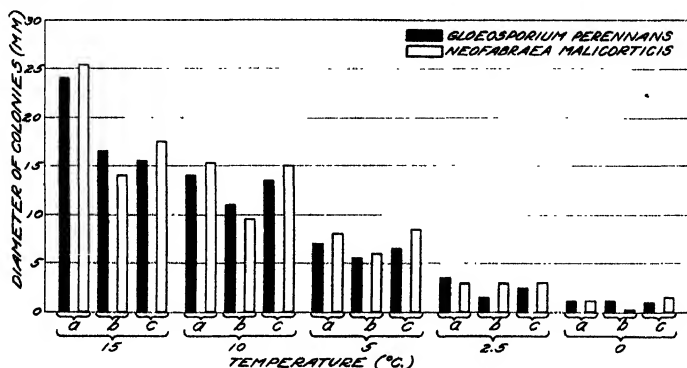


FIGURE 1.—Growth of *Gloeosporium perennans* and *Neofabraea malicortici* at various temperatures on several modifications of Dox's agar in 20 days: a, Dox's agar plus 0.5 per cent dextrose; b, Dox's agar plus 0.5 per cent dextrose less KCl; c, Dox's agar plus 0.5 per cent dextrose less NaNO₃.

up in strict accordance with the directions of Clark (4) both as to purity of chemicals and details of preparation. The buffers were standardized by means of Bunker's modification of the Hildebrandt bubbling hydrogen electrode, a calomel half cell, and a Leeds & Northrup type K potentiometer. A Bailey electrode was employed for determinations in some instances. The accuracy of the apparatus was determined by making readings on N/10 hydrochloric acid and M/20 potassium acid phthalate solutions.

Indicator solutions were prepared as directed by Clark (4), and by means of these and the buffers already mentioned a set of color standards was obtained covering a range of pH 1.2 to 10. For values higher than pH 10, two dyes, Tropaeolin O and Alizarin G, were obtained from the Bureau of Chemistry and Soils. These dyes have been recommended by McCrumb and Kenney (11) for pH values 10 to 13. Although it was hoped to obtain pH values up to 12 or more, it was not deemed advisable to employ any higher than 11.8 because of the similarity of the color of the agar to that of the two dyes. The

pH values were determined colorimetrically by the use of the above-mentioned color standards for comparison.

Very acid or very alkaline agar will not solidify readily, and because of this fact the media having values under pH 3.8 or over 8.2 were sterilized first and the acid or alkali was added later by means of sterile pipettes. Those in the intervening range were combined with the approximate amount of acid or alkali and then sterilized in the autoclave with 10 pounds pressure for 10 minutes. The pH value was determined while the medium was still liquid. The agar was next poured into sterile Petri plates and when cool was inoculated in the center with spores. The plates were held for three weeks at 10° C., and the diameters of the colonies were measured at regular intervals. The results appear in Table 1 and in Figures 2 and 3.

TABLE 1.—Growth of *Neofabraea malicorticis* and *Gloeosporium perennans* on apple agar of different initial pH values after three weeks at 10° C.

Organism	Diameter of colonies (in millimeters) at pH of—																		
	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6	4.8	5.0	5.2	5.4	5.6
<i>Neofabraea malicorticis</i>	0	3.3	4.5	8.0	14.5	24.5	22.0	28.5	28.5	29.0	29.0	28.5	30.0	30.0	30.1	30.0	30.1	29.6	30.8
<i>Gloeosporium perennans</i>	0	1.0	8.2	10.2	14.5	22.2	25.0	27.7	26.5	24.0	33.5	32.0	30.1	31.0	27.1	27.3	26.5	25.0	10.0

In the first of these experiments it appeared that the limiting pH value in the acid range lay between 2.0 and 3.6 for both organisms. Later it was found that the growth rate increased as the pH values were increased by 0.2 pH from 2.2 up to pH 3.0. From pH 3.0 up to pH 11.3 growth was normal. No growth was obtained at pH 2.0. At the alkaline end definite retardation was evinced at pH 11.8. This pH value appeared to exert a greater retarding effect on *Gloeosporium perennans* than on *Neofabraea malicorticis*, but the difference may be entirely experimental, because germination studies indicate that both fungi respond similarly in alkaline media. For instance, in some preliminary tests germination for both organisms was considerably reduced at pH 11.5 and there was no germination at pH 12.

Thus far the physiological responses of these two fungi (*Neofabraea malicorticis* and *Gloeosporium perennans*) have been shown to be very similar. Varying the nutrients in regard to potassium chloride or sodium nitrate did not affect the growth of one organism more than that of the other. The effects of different pH values on the rate of growth were similar for both organisms.

TANNIC ACID MEDIA

For further cultural studies of the two organisms a medium containing tannic acid was employed. Bavendamm (1) recommends a solid medium containing 0.5 per cent tannic acid for the purpose of detecting oxidases in wood-rotting fungi. Inasmuch as the advance of the colony is preceded by a dark ring, due to the oxidasic action, it was hoped that different rates of growth might be easily detected on this medium.

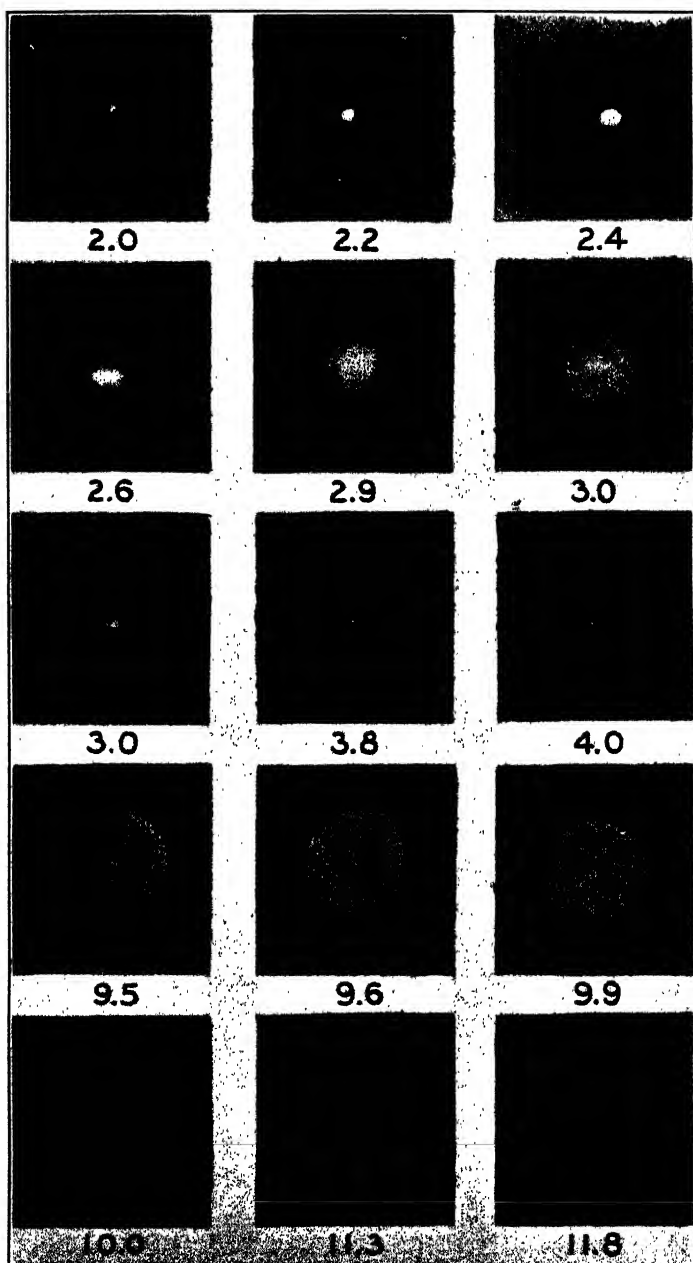


FIGURE 2.—Growth of *Neofabraea malicorticis* in 3 weeks at 10° C. on apple agar of 15 initial pH values from 2.0 to 11.8

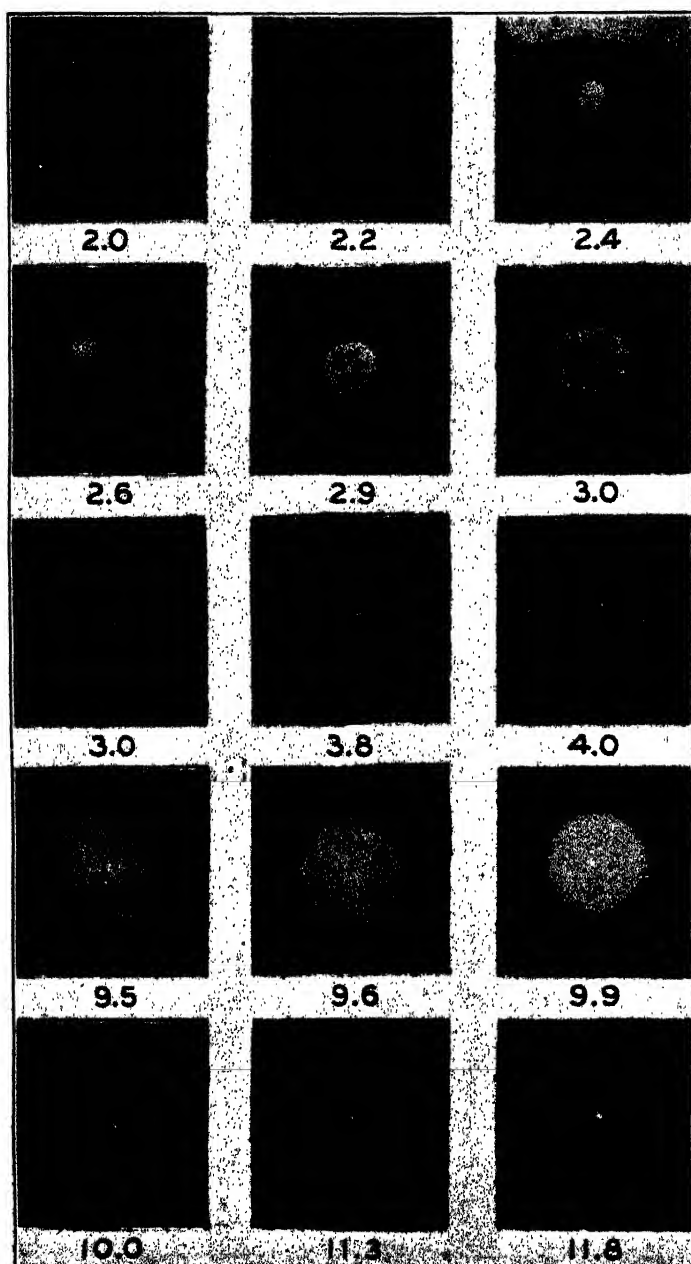


FIGURE 3.—Growth of *Gloeosporium perennans* in 3 weeks at 10° C. on apple agar of 15 different initial pH values from 2.0 to 11.8

A medium containing 4 per cent corn-meal agar and 0.5 per cent tannic acid was inoculated with mycelial tufts of *Neofabraea malicorticis* and *Gloeosporium perennans* and held at 10° C. for 21 days, and at 15° for 18 days. The results appear in Figure 4.

The tannic acid exerts an inhibiting influence on the growth of the two organisms, the effect being more marked for the *Neofabraea malicorticis*. With 0.5 per cent tannic acid the average retardation of

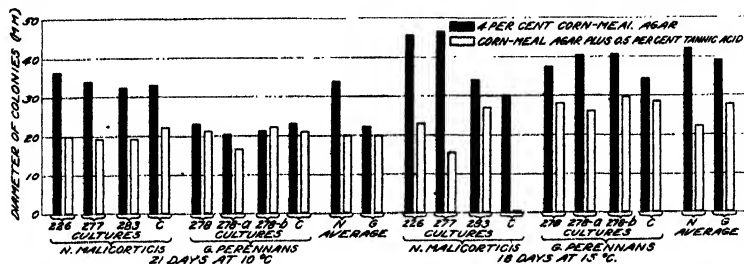


FIGURE 4.—Growth of four cultures each of *Neofabraea malicorticis* and *Gloeosporium perennans* on 4 per cent corn-meal agar and corn-meal agar plus 0.5 per cent tannic acid at 10° and 15° C.

growth for *N. malicorticis* at 10° C. was over three times as great as that for *Gloeosporium perennans*, and at 15° it was more than one and one-half times as great. As great differences were frequently found among strains of the same fungus as between the two fungi: For instance, at 15° C. the reaction of culture 283 of the anthracnose organism was more like that of the perennial canker than some of the cultures of *Neofabraea* (227 or Cooley's).

These studies were continued with the amount of tannic acid increased to 1 per cent. With this percentage the tannic acid usually

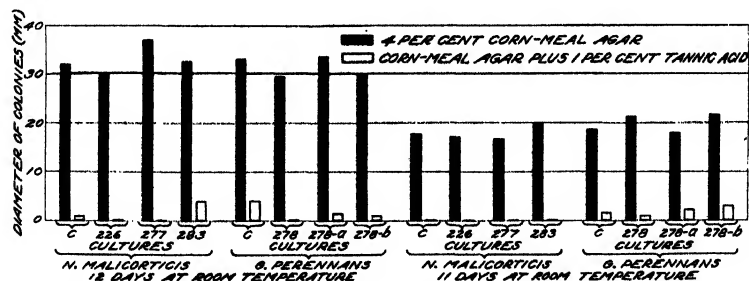


FIGURE 5.—Growth of four cultures each of *Neofabraea malicorticis* and *Gloeosporium perennans* on 4 per cent corn-meal agar and corn-meal agar plus 1 per cent tannic acid at room temperature

settles out after autoclaving, but if the medium is shaken while hot the tannic acid becomes dispersed again and remains so when the medium has become solid.

In 1 per cent tannic acid media the fungi grow with great difficulty at room temperature, there being no visible growth for a week or more. As will be seen from Figure 5, some of the strains had not begun growth at all at the end of 11 and 12 days, those that had started grew very feebly, and it was practically impossible to make any comparison between the effects on the two fungi.

TEMPERATURE STUDIES

Cultures of *Neofabraea malicorticis* and *Gloeosporium perennans* were grown on potato-dextrose agar at the temperatures 0°, 2.5°, 5°, 10°, 15°, and 20° C. The diameters of the colonies were measured at regular intervals. Duplicate readings were averaged and are presented in Figure 6. Within the range studied the increase in growth with increase in temperature was regular for both organisms, but the contrast in temperature responses between the two was no greater than between two cultures of the same organism.

STUDY OF APPLE ROTS

The same six temperatures were employed for a study of the apple rots produced by these two fungi. Temperature relations of apple-

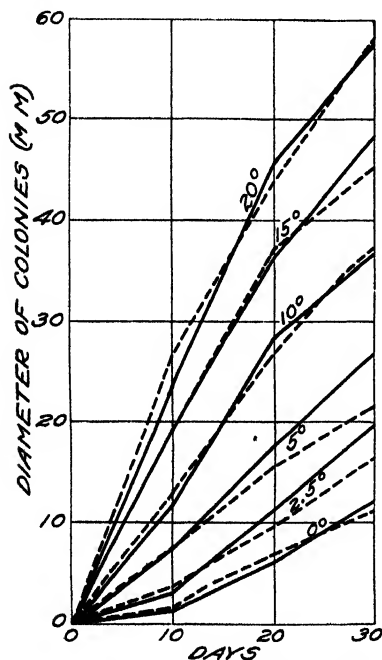


FIGURE 6.—Growth of *Gloeosporium perennans* (solid lines) and *Neofabraea malicorticis* (broken lines) on potato-dextrose agar at different temperatures

rotting fungi have been studied and reported by Brooks and Cooley (2). A number of common organisms attacking apple fruits were studied, among them *Neofabraea malicorticis*. Those writers state that they are not certain whether the culture was *N. malicorticis* or *Gloeosporium perennans*, as the latter had not yet been described but has since been shown to have existed at that time. However, the cultures were obtained from a part of the State of Washington where perennial canker rarely occurs, and in all probability the organism was *N. malicorticis*.

Apples possess several advantages over artificial media in the study of these two fungi. The fungi can not only be made to sporulate on the fruit, but they also produce macrospores more abundantly. On artificial media the microspores are produced in greater abundance. Jackson (10), writing of northwestern anthracnose, states that he frequently noticed the macrospores on apple fruits but was unable to obtain

them by artificial inoculations except on the bark of young apple trees. In the present work apples could generally be relied on for the production of macrospores.

The apples were immersed in 50 per cent alcohol, then in mercuric chloride solution (1 to 1,000), and finally rinsed in hot water. Spores were inoculated into the sides of the apple fruit by means of a sterile needle. The apples were placed in moist chambers and held at the six temperatures heretofore mentioned. The diameters of the rots were measured once a week. The varieties used were Delicious, Grimes Golden, and Winesap. Ten apples were used for each organ-

ism at each temperature in all cases except for Grimes Golden apples, in which only two were used, and for the studies of Zeller's strains on Winesaps, in which only five were used. The results appear in Figures 7, 8, and 9. In Figure 7 the growth of *Gloeosporium perennans* is compared with that of *Glomerella cingulata* (Stoneman) Spaulding and Von Schrenk, the bitter-rot organism of the East.

The two fungi when grown on apples responded to the six temperatures in practically the same manner as they did when grown on artificial media. The growth curves for the two are similar. There was a gradual increase in growth with increase of temperature from 0° to 15° C. Between 15° and 20° the increase was not quite so rapid.

Zeller's three strains of *Gloeosporium perennans* and his three of *Neofabraea malicorticis* were inoculated into Winesap apples and held at the same six temperatures. The results appear in Figure 10. In this case different strains of the same organism grew at different rates, this being more marked with *N. malicorticis* than with *G. perennans*. Strain 283 of *N. malicorticis* made the most rapid growth, with 277 next, and 226 last. With *G. perennans*, strain 278 made the best growth, with 278-b next at temperatures of 5°, 15°, and 20° C. At temperatures of 0°, 2.5°, and 10°, the two strains 278-a and 278-b showed little difference in rate of growth.

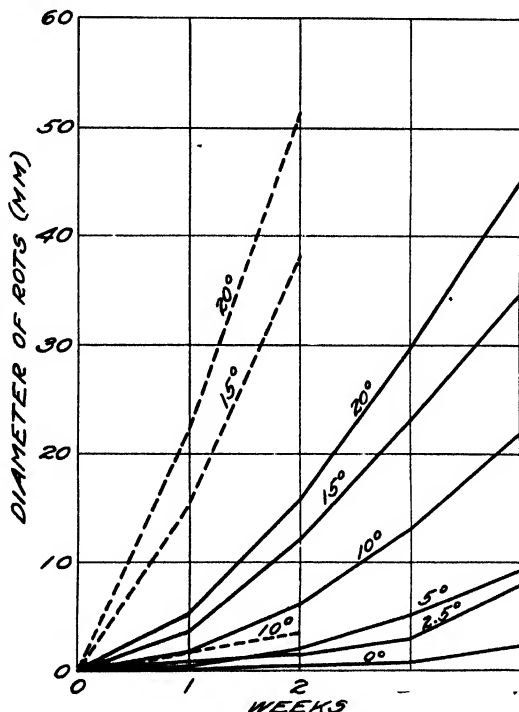


FIGURE 7.—Increase in diameter of rot on Grimes Golden apples produced by *Gloeosporium perennans* (solid lines) and *Glomerella cingulata* (broken lines) in 1 to 4 weeks at various temperatures

GERMINATION OF SPORES

For spore-germination studies apple fruits were inoculated with the two fungi and held at a low temperature until spores were produced. Distilled-water suspensions of the spores were then prepared in Van Tieghem cells and placed at temperatures 0°, 5°, 10°, 15°, 20°, 25°, and 30° C. The percentage of spores germinating, as well as the length of germ tubes, were determined at the end of 24 hours. At least four Van Tieghem cells were employed for each temperature,

and the experiment was repeated several times. In spite of the great number of spores counted there was considerable variation in percentage of germination, this being particularly true at 20°. The graphs in Figure 11 represent average percentage of germination based on the mass of evidence obtained.

Spores of both fungi failed to germinate at 0° C. within 24 hours, although they were found to have started at the end of 48 hours. Germination increased with rise of temperature from 0° to 15°, and the optimum appeared to be in the range of 15°, 20°, and 25°, with no great differences between any of these temperatures for either fungus. At 30° there was practically no germination for either fungus.

The increase in length of germ tube was consistent with increase of temperature up to 20° C. In Figure 12 it will be

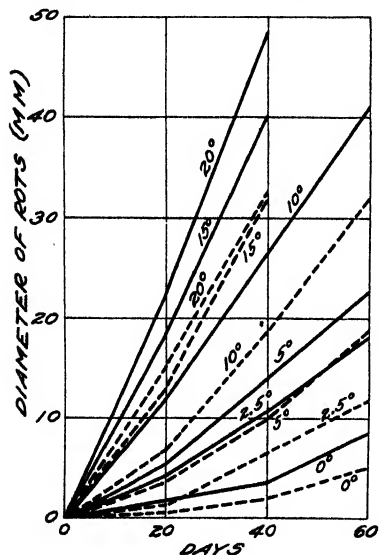


FIGURE 8.—Increase in diameter of rots on Delicious apples produced by *Gloeosporium perennans* (solid lines) and *Neofabraea malicorticis* (broken lines) in 20, 40, and 60 days at various temperatures

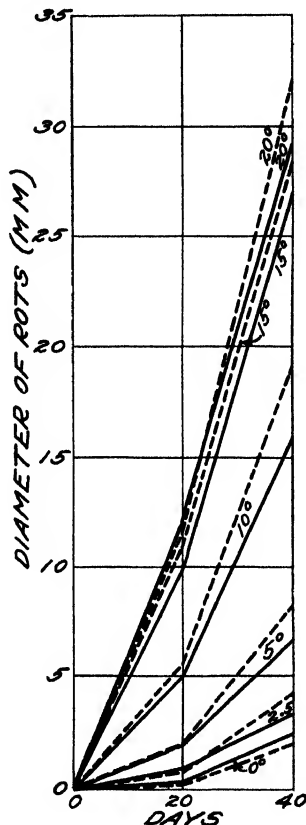


FIGURE 9.—Increase in diameter of rots on Winesap apples produced by *Gloeosporium perennans* (solid lines) and *Neofabraea malicorticis* (broken lines) in 20 and 40 days at various temperatures

noted that the rate of growth declined at 25°. This applies to both organisms.

SUMMARY AND CONCLUSIONS

Temperature responses of the two apple-rotting and canker fungi, *Neofabraea malicorticis* and *Gloeosporium perennans*, were very similar, whether inoculated into artificial media or into apple fruits. Both exhibited increased growth with rise of temperature in the range of 0° to 20°C., the rate of increase on apples falling off slightly from

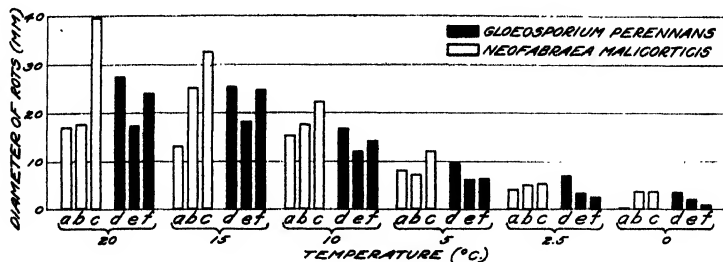


FIGURE 10.—Increase in diameter of rots on Winesap apples produced by three strains each of *Neofabraea malicorticis* and *Gloeosporium perennans* in 34 days at various temperatures. *Neofabraea* strains—*a*, 226; *b*, 277; *c*, 283. (*Gloeosporium* strains—*d*, 278; *e*, 278-*a*; *f*, 278-*b*)

15° to 20°. Both germinated and grew at 0° and rotted fruit at that temperature. Macrospores were readily obtained from inoculated apple fruits, being very abundantly produced at 10°.

In such nutritional studies as have been made, the two fungi have shown similar responses. The absence of potassium chloride or sodium nitrate from the nutrients in Dox's agar appeared to exert slight retarding effect on the

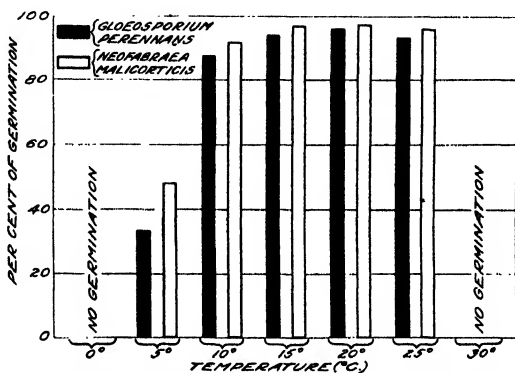


FIGURE 11.—Germination of *Gloeosporium perennans* and *Neofabraea malicorticis* in distilled water in 24 hours at various temperatures

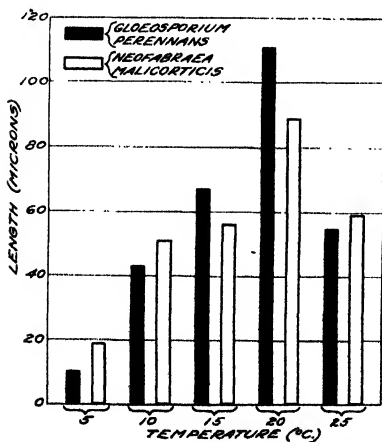


FIGURE 12.—Growth of germ tubes of *Gloeosporium perennans* and *Neofabraea malicorticis* in 24 hours at various temperatures

growth of either organism. Gregory and Horne (9), working with other fungi, report that in the invasion of the apple fruit high resistance is associated with low water and low nitrogen content and high potash and high acidity. The present work is hardly comparable with that of Gregory and Horne, because their experiments concerned the apple fruit rather than artificial media, and they were studying different fungi.

With regard to the hydrogen-ion studies, it is evident that both fungi experience difficulty in growing at a pH of less than 3.0 and can not grow at all at a pH of 2.0. In the alkaline range a pH of 11.8 produced definite inhibitory effects on both fungi.

Though the inhibition appeared slightly greater for *Gloeosporium perennans* than for *Neofabraea malicorticis*, germination studies indicate that there is no great difference in the responses of the two fungi in this range. It is realized that the organisms probably change the pH of the medium during growth and that the results indicate the ability of the fungi to make initial growth in the media of the various pH values. However, definite retardation of initial growth was noted in pH values of less than 3.0 and at 11.8, and pH values of 2.0 and 12.0 are the points where even initial growth can not be made.

The outstanding effect of the tannic acid medium is the retardation of growth of *Neofabraea malicorticis*. In some instances the fungus barely started growth by the end of 12 days; in others it was able to grow in the tannic acid medium, but the rate of growth was always retarded. From the results in Figure 4 it will be noted that the retardation is much more pronounced for *Neofabraea* than for *Gloeosporium*.

Optimum temperatures for germination of spores were found to be in the range of 15°, 20°, and 25° C. Though there was no germination at 0° in 24 hours, spores of both fungi were producing germ tubes in 48 hours, and a count revealed a germination of 34 to 46 per cent. At 30° there was no germination in 72 hours.

In the physiological studies conducted on *Neofabraea malicorticis* and *Gloeosporium perennans* there was greater variability among the different strains of the same species than between the two fungi themselves.

LITERATURE CITED

- (1) BAVENDAMM, W.
1928. ÜBER DAS VORKOMMEN UND DEN NACHWEIS VON OXYDASEN BEI HOLZZERSTÖRENDENDEN PILZEN. Ztschr. Pflanzenkrank. u. Pflanzenschutz. 38:257-276, illus.
- (2) BROOKS, C., and Cooley, J. S.
1917. TEMPERATURE RELATIONS OF APPLE-ROT FUNGI. Jour. Agr. Research 8:139-164, illus.
- (2a) BROWN, N. A.
1932. SOME PATHOLOGICAL STUDIES ON APPLE CANKERS. Phytopathology 22:397-414, illus.
- (3) CHILDS, L.
1927. PERENNIAL CANCER OF APPLES. Oreg. State Hort. Soc. Ann. Rpt. 19:108-117.
- (4) CLARK, W. M.
1920. THE DETERMINATION OF HYDROGEN IONS; AN ELEMENTARY TREATISE ON THE HYDROGEN ELECTRODE, INDICATOR AND SUPPLEMENTARY METHODS, WITH AN INDEXED BIBLIOGRAPHY ON APPLICATIONS. 317 p., illus. Baltimore.
- (5) ——— and LUBS, H. A.
1916. HYDROGEN ELECTRODE POTENTIALS OF PHTHALATE, PHOSPHATE, AND BORATE BUFFER MIXTURES. Jour. Biol. Chem. 25:479-510, illus.
- (6) CORDLEY, A. B.
1900. APPLE TREE ANTHRACNOSE; A NEW FUNGOUS DISEASE. Oreg. Expt. Sta. Bul. 60, 8 p., illus.
- (7) FISHER, D. F.
1925. A NEW APPLE ROT DISEASE. ("BULL'S-EYE ROT"—CAUSED BY GLOE-OSPORIUM, ZELLER AND CHILDS). Wash. State Hort. Assoc. Proc. 21:44-48.
- (8) ——— and REEVES, E. L.
1928. PERENNIAL CANCER. Wash. State Hort. Assoc. Proc. 24:55-61, illus.

- (9) GREGORY, F. G., and HORNE, A. S.,
 1928. A QUANTITATIVE STUDY OF THE COURSE OF FUNGAL INVASION OF THE
 APPLE FRUIT AND ITS BEARING ON THE NATURE OF DISEASE RESIST-
 ANCE. PART I. A STATISTICAL METHOD OF STUDYING FUNGAL
 INVASION. Roy. Soc. [London] Proc., Ser. B 102:427-466, illus.
- (10) JACKSON, H. S.
 1913. APPLE TREE ANTHRACNOSE. A PRELIMINARY REPORT. Oreg.
 Expt. Sta. Crop Pest and Hort. Rpt. 1911-12:178-197, illus.
- (11) McCURMB, F. R., and KENNY, W. R.
 1929. AN EXAMINATION OF POSSIBLE INDICATORS TO DETERMINE THE pH
 OF ALKALINE SOLUTIONS. Indus. and Engin. Chem., Analyt.
 Ed. 1:44-46.
- (12) PECK, C. H.
 1900. NEW SPECIES OF FUNGI. Bul. Torrey Bot. Club 27:14-21.
- (13) ZELLER, S. M., and CHILDS, L.
 1925. PERENNIAL CANCER OF APPLE TREES (A PRELIMINARY REPORT).
 Oreg. Agr. Expt. Sta. Bul. 217, 17 p., illus.

MACROCENTRUS ANCYLIVORUS ROH., AN IMPORTANT PARASITE OF THE ORIENTAL FRUIT MOTH¹

By G. J. HAEUSSLER²

Assistant Entomologist, Division of Fruit and Shade Tree Insects, Bureau of Entomology, United States Department of Agriculture

INTRODUCTION

An investigation of the oriental fruit moth (*Laspeyresia*) *Grapholitha molesta* (Busek), and its parasites, conducted at Riverton, N. J., in 1925 by Peterson and Haeussler (4)³, showed that the very considerable reduction of the infestation by this pest in peach fruit was brought about chiefly by one species of parasite, a braconid of the genus *Macrocentrus*. Further studies by the writer (3), conducted in the same district from 1925 to 1928, showed that a great reduction in the population of the midsummer and late broods of twig-infesting larvae resulted each season from the severe parasitism by *Macrocentrus* in the larvae of the earlier broods. The average parasitism by this braconid in larvae collected from twigs ranged from 41 to 55 per cent for the four seasons. In 1925 and 1926, collections of larvae which were feeding in twigs during late June and early July showed parasitism by this one species as high as 92 and 84 per cent.

Cross parasitism and cross-breeding experiments by Stearns (8) and by the writer showed this parasite to be identical with that reared by Fink (2) from the strawberry leaf roller (*Ancylys comptana* Froel.) and described by Rohwer (7) as *Macrocentrus ancylivora*. The experiments consisted in the securing of parasitism of each host species by parasites reared from the other and the mating of female parasites from one host with males from the other, with subsequent propagation on both host species.

Because of the importance of this species as a parasite of the oriental fruit moth, it seemed advisable to make a detailed study of the species in its relation to this host. Its biology as a parasite of the strawberry leaf roller has been reported by Fink (2), and Stearns (8) published on its biology with reference to both *Grapholitha molesta* and *Ancylys comptana* as hosts. The present study deals entirely with the development of this parasite when bred from the oriental fruit moth.

Preliminary investigations on methods of handling the parasite under insectary conditions and a study of the habits of the species were begun in 1926. The more detailed study was taken up at Riverton in 1927, the insectary studies being started with the first adult parasites that emerged from the earliest parasitized fruit-moth larvae collected in the orchards that spring. In November, 1927, the insectary was moved from Riverton to Moorestown, N. J., a

¹ Received for publication Jan. 12, 1932; issued August, 1932.

² The writer expresses his indebtedness to Alvah Peterson, formerly of the Bureau of Entomology of the U. S. Department of Agriculture, under whose direction this study was begun, for valuable advice, and to S. A. Rohwer, R. A. Cushman, and A. B. Gahan, of the Bureau of Entomology, for the determination of specimens. This report is based on investigations conducted prior to July, 1929, when the author was assigned to work in France.

³ Reference is made by number (italic) to Literature Cited, p. 100.

distance of about 5 miles, and the work was continued at Moorestown until the last hibernating individuals had emerged in the spring of 1929.

METHODS USED IN STUDYING THE PARASITE

The following methods were used in conducting the study of *Macrocentrus ancylivorus* under insectary conditions with *Grapholitha molesta* as a host: *Grapholitha* larvae, originating from eggs deposited upon pear leaves in the insectary, were allowed to begin their development by feeding in apples. When the larvae were from 4 to 8 mm long they were removed from the fruit as needed and placed



FIGURE 1.—Apparatus used for obtaining parasitism of larvae of *Grapholitha molesta*

on young, succulent peach twigs. The stems of the twigs were first inserted in a small bottle of water and held in a tight bunch by means of a cotton plug in the neck of the bottle. (Fig. 1.) A bunch of 8 or 10 twigs was found to accommodate from 10 to 15 larvae satisfactorily. Within an hour or two the larvae would spin a light silken web about their bodies to hold themselves against the twigs while eating their way in. The larvae were then in suitable position for being parasitized, and the bottle of twigs was placed in a cage containing parasites of both sexes. Late in the season, when young, succulent peach twigs were no longer available, good results

were obtained by placing the partly grown larvae on cross-section slices of apple, about one-eighth inch thick. The larvae soon ate their way into the soft tissue where, owing to the thinness of the slices, the ovipositors of the female parasites could easily reach them when the slices were suspended on wire hooks in the parasite cages. The type of cage which proved most satisfactory for obtaining parasitism under insectary conditions is shown in Figure 1. This cage was 6 inches square and 8 inches high, with a solid wooden base and wooden framework covered on three sides and the top with 24-mesh galvanized wire screen. The entire fourth side was a sliding door of heavy transparent celluloid which could be raised for inserting or removing the bottle of twigs or the parasites. A glass castor cup containing a piece of sponge saturated with water was placed in the cage to provide moisture for the parasites. On extremely hot dry

days wet cheesecloth, draped over the top and the side of the cage opposite the door, aided materially in prolonging the life of the adult parasites. A high percentage of parasitism resulted when the twigs on which larvae were feeding remained in this cage from 4 to 24 hours, depending upon the number of female parasites present and the prevailing temperature. As a rule, the larvae were kept in the cage for 24 hours.

After the bottle of twigs was taken out of the cage, the twigs were removed and the lower parts of the stems which had been submerged in the water were cut off. The portions of twigs containing the larvae were placed in a jelly glass with one or more apples which provided additional food for the larvae when the twigs had become too dry. A narrow strip of corrugated paper was also placed in the glass, which was then covered with cheesecloth held tightly in place by rubber bands. (Fig. 2.)

When the larvae finished feeding and became full grown they left the fruit and spun their cocoons, usually in the corrugated paper. The glasses were examined once daily, and each corrugation containing a cocoon was cut out, given a record number, and placed in a small homeopathic vial until the adult emerged. Any full-grown larvae found crawling about the glass at the time of examination were placed in individual 2-dram vials stoppered with cloth-covered cotton plugs. These larvae spun their cocoons against the glass, and observations could readily be made of subsequent development without the necessity of disturbing them in any way. Each of these vials was examined once daily, and records were made for every individual whenever any important change occurred. Since it is usually only a matter of a few hours from the time the parasite larva emerges from the body of its host until it spins a cocoon of its own, the emergence of the parasite larva from its host was missed in many instances in the daily observations. Consequently, some of the vials were examined several times a day in order to determine the length of time that the larvae of *Macrocentrus* feed externally on the host. If a fruit-moth larva pupated it was known to be unparasitized, and subsequent records for that individual pertained to normal fruit-moth development.

For each individual reared to maturity in observation vials, the dates of moth egg deposition, parasite egg deposition, moth cocoon formation, parasite cocoon formation, pupation of unparasitized fruit

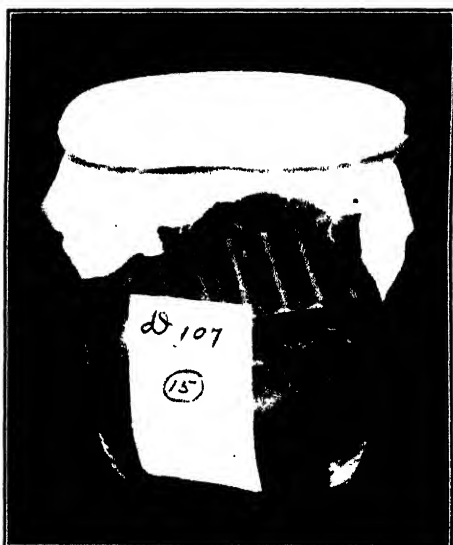


FIGURE 2.—Jelly glass in which parasitized larvae continued feeding

moths, and adult emergence of either parasite or moth were recorded. Thus it was possible to compute the length of the life cycle as well as the length of several of the developmental periods for each individual that emerged, whether *Macrocentrus* or *Grapholitha*.

THE ADULT

DESCRIPTION

The following description of the adult of *Macrocentrus ancylivorus* (fig. 3) is largely a condensation of the original description by Rohwer (7) and subsequent descriptions by Fink (2) and Stearns (8).



FIGURE 3.—Adults of *Macrocentrus ancylivorus*: A, Female; B, male. $\times 4.5$

The female averages about 4.5 mm in length, the ovipositor 5.5 mm, antenna 7.5 mm, and wing expanse about 9 mm, male often slightly smaller; General body color pale ferruginous; eyes and ocelli black; antennae uniform dark brown distad of the pedicel, segmentation not clearly distinct except with a strong lens; base and apex of abdomen of both sexes sometimes marked with dark brown; face, ventral part of antennal scape and pedicel, ventral part of thorax, and sometimes venter of abdomen lighter colored, almost yellow; legs yellow.

SIMILARITY TO *MACROCENTRUS DELICATUS* CRESS.

In 1926, a few lighter colored adults of *Macrocentrus* were noted among those emerging from field-collected material. At first these were considered as merely color variations of the one species, but all such individuals were noted in the records, and the specimens were preserved. Similar adults were obtained in 1927, and, since these emerged each season from larvae that had been collected over a period of about two weeks during late June and early July, it was suspected that they might be of a separate species. Mating was never observed between light-colored individuals of one sex and dark-colored individuals of the opposite sex, whereas both light and dark individuals mated readily with similar adults of the opposite sex. It was further noted in 1927 that dark-colored females which had been fertilized always produced dark-colored progeny and that fertile light-colored females always produced light-colored progeny. R. A. Cushman, of the taxonomic unit of the Bureau of Entomology, later determined the light-colored specimens to be a distinct species, *M. delicatus* Cress.

Morphological differences occurring in the two species, as well as characters useful in distinguishing adults of *ancyliivorus* from those of *delicatus*, have been very fully discussed by Driggers and Pepper (1). The writer found the difference in appearance of the antennae and the difference in general body color to be the most useful characters for distinguishing living adults of one species from those of the other species. That the antennae of *delicatus* are longer and have a segmented or "beaded" appearance whereas those of *ancyliivorus* are shorter and are "hairlike" in appearance, as mentioned by the authors cited, was noted by the writer in 1927 and used at that time as a distinguishing character.

MATING

When adults of both sexes of *Macrocentrus ancyliivorus* are confined together the male usually attempts mating almost immediately. The wings of the male are raised almost perpendicularly and flutter rapidly as he persistently follows the female. Upon approaching close to the female from the rear the male curls the tip of the abdomen forward from beneath in an endeavor to reach the female. The latter often flies out of reach, and it is sometimes necessary for the male to make several attempts before mating is accomplished.

For 18 observed matings the minimum, average, and maximum duration of the act was 12, 22.5, and 45 seconds, respectively. After mating, the female usually repels further attempts on the part of the same or other males, although in one instance when one female was confined in a 6 by 8 inch glass jar with three males the female was observed mating four distinct times for 30, 12, 19, and 25 seconds, with intervals of 4, 1½, and 11 minutes, respectively. The last three matings were with the same male. A male may mate with several females; thus when more females than males are placed in a cage most of the females usually become fertilized. Mating activity is most noticeable in the cages on warm summer days and during the warmer hours of the day in spring and fall.

PROPORTION OF SEXES PRODUCED

More females than males of *Macrocentrus ancyliivorus* are recovered from larvae of the oriental fruit moth parasitized normally in the orchards in the vicinity of Riverton, N. J. For the three consecutive seasons, 1926, 1927, and 1928, the percentage of female parasites which emerged from parasitized larvae collected in infested peach twigs was consistently greater than the percentage of males. During the respective seasons, 55.2, 55.5, and 53.4 per cent of the parasites produced were females. A total of 2,642 adult parasites were reared from fruit-moth larvae parasitized in the field over the 3-year period. Of these, 1,443, or 54.6 per cent, were females and 1,199, or 45.4 per cent, were males.

A greater proportion of female parasites was also obtained from larvae of the strawberry leaf roller parasitized in the field in the same region. Collections of larvae of this host species in 1927 and 1928 produced 58.1 and 58.8 per cent female parasites during the respective seasons. From the total of 219 parasites that emerged during the two years, 128, or 58.4 per cent, were females and 91, or 41.6 per cent, were males.

A consistently greater proportion of females, such as occurs under natural conditions, is not always produced when this species is bred under insectary conditions. Of the total number of parasites reared from *Grapholitha* in the insectary in 1927 in connection with the life-history studies, 64.4 per cent were females. In similar rearings in 1928 only 48.8 per cent were females. However, from the total of 1,562 parasites bred from this host under insectary conditions during the two seasons 894, or 57.2 per cent, were females and 668, or 42.8 per cent, were males.

For securing parasitism of larvae in the insectary, parasites of both sexes were always placed in the oviposition cages. However, it is quite possible that in some instances parasitism occurred before all of the female parasites had been fertilized. Very probably this happened on several occasions in 1928 when, owing to a shortage of males, more female than male parasites were placed in the oviposition cages. This may explain, in part at least, the high percentage of male parasites that was produced during that season, since unfertilized eggs of *Macrocentrus ancylivorus* yield males only.

LONGEVITY

Twenty-two separate experiments were conducted during the season of 1927 to determine the length of adult life when parasites of both sexes were confined together in cages such as those previously described, with either fruit-moth larvae or larvae of the strawberry leaf roller. Records were obtained from a total of 115 female and 79 male parasites. The minimum, average, and maximum length of life for both sexes confined together was 1 day, 6.7 days, and 14 days, respectively. The highest average longevity for the females in any single experiment was 11.1 days, for the males 11.3 days, and for the two sexes together 10.6 days.

For eight unfertilized females that were confined in a cage without males, but provided with *Grapholitha* larvae in which to oviposit, and fed a 10 per cent solution of honey water, the minimum, average, and maximum length of life was 13, 17.9, and 29 days. When freshly emerged males were confined in a cage with no females and no larvae present, but fed the 10 per cent solution of honey water, the respective duration of life was 7, 10.3, and 19 days.

OVIPOSITION

Oviposition by *Macrocentrus ancylivorus* on *Grapholitha molesta* is most readily accomplished when the larva of the host species is in a position from which it can not easily escape the thrusts of the ovipositor of the female parasite. The most favorable position for attack seems to be when the larva is feeding within a peach twig or a thin slice of apple, or is inclosed in a light feeding web preparatory to entering a twig. Successful oviposition has never been observed by the writer when the host larva was not in some such fixed position. If free to escape, the larva wriggles out of reach at the first touch of the ovipositor and thus frightens the parasite.

When a number of full-grown larvae of the oriental fruit moth were killed in hot water, inserted in nail punctures in a piece of dried corn-stalk, and placed in an oviposition cage, female parasites oviposited readily in the bodies of the dead larvae. The dead hosts dried up,

however, before the parasite eggs could develop. On several occasions females were observed endeavoring to parasitize full-grown *Grapholitha* larvae that were wandering about the cage or other container, but the larvae always managed to escape the thrust of the ovipositor, and in no instance was such attempted parasitization successful.

Females of this species have been observed ovipositing at practically all hours of the day during warm summer weather. On several occasions, when a flash light was suddenly turned on the oviposition cages after dark, parasites were observed ovipositing as late as 10 p. m.

Under insectary conditions a female parasite will oviposit several times in the same larva. One female was observed to puncture a larva 17 times and another female oviposited 13 times in a single larva. Dissections of 12 hibernating *Grapholitha* larvae which had been parasitized in cages in the insectary showed that, although superparasitism occurs, only one parasite larva survives regardless of the number of eggs deposited. Each host larva contained one living parasite larva. In 11 of the host larvae from 1 to 58, or a total of 131, head capsules and bodies of dead parasite larvae were also present. The remaining host larva contained no sign of superparasitism.

PARTHENOGENESIS

Unfertilized females can deposit eggs that will mature, but the progeny are always males. In 1927 a cage containing 10 freshly emerged unfertilized females was started on July 28. From numerous insectary-bred larvae of the oriental fruit moth which were placed in this cage a few at a time, a total of 63 parasites was produced, all of them males. When some of these males were allowed to mate with unfertilized females emerging from larvae of the same host that had been parasitized in the field, normal progeny of both sexes were produced.

STAGE OF HOST ATTACKED

Macrocentrus ancyliivorus will attack the feeding larvae of the oriental fruit moth in any stage of development, from the very small first-stage larvae to those that are nearly full grown. Table 1 shows the parasitism by this species which occurred in *Grapholitha* larvae of various sizes taken in weekly collections of infested twigs from orchards near Riverton during the season of 1927. Each larva was removed from the twig within an hour after it had been collected, and its length in millimeters was recorded. The larvae were divided into three groups, according to length, and placed on apples in jelly glasses to continue their development. One group contained larvae measuring from 1.5 mm (recently hatched) to 3.5 mm, inclusive, another group contained those measuring over 3.5 to 6.5 mm, inclusive, and the third group contained those measuring over 6.5 mm. Practically all the larvae of the third group were in the last larval instar. The table shows, for each respective size group, the number of *Grapholitha* larvae collected, the total number of individuals that completed development and emerged as adults (including moths and all species of parasites), and the number of adult *M. ancyliivorus* that emerged. The percentage of all individuals that reached maturity, based on the number of larvae collected, and the percentage of *M. ancyliivorus* that emerged, based on the total emergence, are also given for each of

the three groups and for the combined total. It will be noted that the percentage of individuals reaching maturity increased decidedly with each increase in the size of the larvae when collected. This is explained, in part at least, by the fact that more of the smaller larvae were injured in handling and failed to enter the new food medium after having been removed from the twigs. There was also an increase in the percentage of *Macrocentrus* that emerged from the larvae of each progressive size.

TABLE 1.—Numbers and percentages of individuals reared to maturity and *Macrocentrus ancyliivorus* adults emerged from *Grapholitha molesta* larvae of various sizes taken in field collections of infested peach twigs at Riverton, N. J., 1927

Grapholitha larvae		Adults, both moths and all parasites, emerging		Macrocentrus ancyliivorus emerging	
Size when collected	Collected				
	Number	Number	Per cent	Number	Per cent ^a
1.5 to 3.5 mm.....	900	193	21.44	97	50.26
Over 3.5 to 6.5 mm.....	974	361	37.06	193	53.46
Over 6.5 mm.....	1,015	651	64.14	379	58.22
Total or average.....	2,880	1,205	41.71	669	55.52

^a Based on total emergence of moths and parasites.

Under insectary conditions it was also found that the percentage of individuals reaching maturity was greater with each increase in the size of the larvae at the time these were transferred from apples to twigs before being placed in the parasite oviposition cages. The majority of the larvae used in the life-history studies ranged in length from 4 to 8 mm, inclusive. During both 1927 and 1928, fruit-moth larvae ranging from 4 to 6.5 mm in length were more heavily parasitized than those measuring 7 mm and over. The percentages of parasites that emerged from a considerable number of larvae that had been separated into these two size groups were 56.98 and 47.96, respectively, in 1927, and 83.33 and 70.81, respectively, in 1928. There were not sufficient numbers of larvae measuring from 1 to 3.5 mm used in the insectary studies to provide comparative data on the percentage of parasitism occurring in these smaller larvae. Of the total number of adults reared from all larvae subjected to the attack of *Macrocentrus* in the insectary in 1927, 1928, and in both seasons combined, 54.72, 71.10, and 61.23 per cent, respectively, were parasites.

Parasitism of *Grapholitha molesta* in the field is confined largely to those larvae of the first, second, and a portion of the third broods which feed within the peach twigs, for when larvae of the late broods enter the fruit it is difficult for the female *Macrocentrus* to reach them with her ovipositor. In 1925 a large number of larvae of the oriental fruit moth were removed from Elberta peaches at harvest time. From these larvae, 627 moths and only 1 *M. ancyliivorus* emerged. The parasitism by this species in larvae that had infested the twigs in that same orchard earlier in the season was very high.

This parasite does not attempt to attack full-grown *Grapholitha* larvae that have formed cocoons, nor does it attempt to oviposit in naked *Grapholitha* pupae.

REPRODUCTIVE CAPACITY

Four females of *Macrocentrus ancyliivorus* were mated within a few hours after they had emerged and were then placed in cages, only one parasite to a cage, and each was provided with 50 larvae of the oriental fruit moth feeding on bundles of twigs. A new lot of 50 larvae was placed in each cage every second day until the parasite died. The four females lived 8, 4, 5, and 6 days; and the number of progeny produced by each individual was 40, 28, 29, and 22, respectively, 119 in all, or an average of 29.75 each.

LIFE HISTORY AND COMPARATIVE RATE OF DEVELOPMENT OF THE PARASITE AND THE HOST

The writer had planned to make a detailed study during the season of 1929 of that period in the development of *Macrocentrus ancyliivorus* during which the parasite lives within the body of the host larva. Circumstances, however, prevented continuation of investigations with this parasite after the spring of 1929. Consequently, information is not available regarding the duration of the egg stage and the number and duration of the larval instars. Data concerning the minimum, average, and maximum number of days required for the several periods of development and for the complete life cycle of *M. ancyliivorus* and its host *Grapholitha molesta* during 1927 and 1928 and for the two seasons combined are presented in Table 2. These data are recorded separately for transforming⁴ and for wintering⁴ individuals. In each instance the number of parasites or fruit moths from which observations were made is indicated.

⁴ The terms "transforming" and "wintering" are used here in the same sense in which they were used by Peterson and Haeussler (6) in their life-history study of the host species. "Transforming" refers to those individuals which, completing their life cycle, transform to adults the same season the eggs are deposited; "wintering" refers to those individuals which hibernate and do not become adults until the following season.

TABLE 2.—Average, minimum, and maximum number of days required for several periods of development and for the life cycle of *Macrocentrus ancyliorinus* and *Grapholitha molesta* in 1927 and 1928, and the number of individuals on which data are based, Riverton and Moorstown, N. J.

MACROCENTRUS ANCYLIORINUS

Period of development	Time required for development						Individuals observed					
	Transforming			Wintering			Transforming			Wintering		
	Two years combined			Two years combined			1927			1928		
	Aver- age	Min- imum	Max- imum	Aver- age	Min- imum	Max- imum	Days	Days	Days	Days	Days	Days
Parasite egg deposition to formation of host cocoon.....	8.3			10.5								
Host cocoon formation to emergence of parasite larva.....	7.8			6.0								
Emergence of parasite larva to formation of host cocoon.....	6	5	4	5	5	4	9.4	9.4	9.4	9.4	9.4	9.4
Formation of cocoon to emergence of adult.....	16.4	14.0	18.2	15.2	13.1	17.1	15.2	15.2	15.2	15.2	15.2	15.2
Life cycle (including cocoons in corrugated paper).....	33.1	31.1	35.1	32.1	30.1	34.1	32.1	32.1	32.1	32.1	32.1	32.1
Life cycle (including cocoons in corrugated paper).....	32.9	29.7	36.1	31.2	28.0	34.4	31.2	31.2	31.2	31.2	31.2	31.2

GRAPHOLITHA MOLESTA

Period of development	Time required for development						Individuals observed					
	Transforming			Wintering			1927			1928		
	Two years combined			Two years combined			1927			1928		
	Aver- age	Min- imum	Max- imum	Aver- age	Min- imum	Max- imum	Days	Days	Days	Days	Days	Days
Egg deposition to formation of cocoon.....	22.0			28.2								
Formation of cocoon to pupation.....	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9	3.9
Pupation to emergence of adult.....	11.6	9.3	10.6	10.6	9.3	11.6	10.6	10.6	10.6	10.6	10.6	10.6
Life cycle (observation vials only).....	37.5	41.4	39.2	39.2	37.5	41.4	37.5	37.5	37.5	37.5	37.5	37.5
Life cycle (including cocoons in corrugated paper).....	35.7	37.9	36.3	36.3	35.7	37.9	35.7	35.7	35.7	35.7	35.7	35.7

* Approximation.

† "Life cycle" includes the complete development, from deposition of the egg to emergence of the adult.

DEVELOPMENT OF THE PARASITE LARVA WITHIN THE HOST

The parasite larva which hatches from the egg deposited within the body of the partly grown *Grapholitha* larva spends the greater portion of its larval life as an internal parasite. When the *Grapholitha* larva has finished feeding in its last stage and reached the quiescent period within its cocoon the parasite larva, having attained its last instar, makes its way out of the host. During the two seasons of 1927 and 1928 (combined), unparasitized transforming *Grapholitha* required from 14 to 51 days, or an average of 24.6 days, from the time the egg was deposited until the cocoon was spun; and unparasitized wintering *Grapholitha* required from 13 to 89 days, or an average of 35 days. Parasite eggs which produced parasites transforming during the same season were deposited in the host larvae from 2 to 31 days, or an average of 9.4 days, before the formation of the host cocoon; and those producing wintering parasites were deposited from 7 to 44 days, or an average of 16.7 days, before the formation of the *Grapholitha* cocoon.

Transforming fruit-moth larvae, when unparasitized, pupate in from 1 to 13 days, or an average of 3.9 days after the cocoon has been spun. The transforming parasite larva emerges from the body of the *Grapholitha* larva in from 1 to 23 days, or an average of 6.9 days, after the host larva has constructed its cocoon. Therefore, the transforming parasite larva emerges from the body of the host on an average of 3 days after the fruit-moth larva would normally have pupated.

Wintering parasite larvae remain within the body of the hibernating host larvae throughout the winter.

The hibernating larva of *Macrocentrus ancylivorus* (fig. 4) averages 2.63 mm in length and 0.43 mm in width. The heavily sclerotized, light-brown head measures 0.24 mm in length by 0.27 mm in width and bears a pair of very strong, heavily sclerotized mandibles. The body, which is of a dirty yellowish white, consists of 13 segments besides the head and is terminated by three hooklike prolongations of the anal segment. The hibernating larvae coincide in all respects with the description given by Fink (2) of the mature first-instar larva of this species.

In the spring, the hibernating larvae attain their last larval instar as internal feeders and then come out of the body of the host, the first parasite larvae appearing externally a few days after the first unparasitized fruit-moth larvae have pupated. Parasite eggs which produce hibernating parasites are deposited later in the fall than are *Grapholitha* eggs which produce hibernating individuals. The average length of time from formation of the host cocoon in the fall to emergence of the parasite larva the following spring (190.3 days) is considerably shorter than the period from the formation of the cocoon to the transformation to pupa (202.8 days) for unparasitized wintering individuals of the host species. Fruit-moth larvae that are parasitized by *M. ancylivorus* do not pupate, as the host larva is always destroyed previous to pupation.

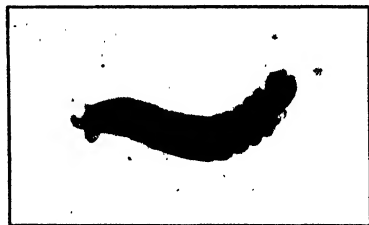


FIGURE 4.—Hibernating larva of *Macrocentrus ancylivorus*. $\times 12.5$

DESTRUCTION OF THE HOST

When the parasite larva comes out of the host it lies parallel to the body of the host larva with its head embedded in the hole from which it emerged. It then proceeds to feed on its former host until



FIGURE 5.—Partly grown *Macrocentrus* larva feeding on a larva of the oriental fruit moth. $\times 5$

nothing remains but the sclerotized parts and the shriveled skin. During warm summer weather the average transforming parasite larva finishes feeding in from about 12 to 18 hours after emerging from its host. Wintering parasites, the larvae of which emerge from the host during cool spring weather, require an average of about 2 days to complete their

feeding. While feeding externally, the dirty yellowish-white parasite larva shows up distinctly against the darker grayish pink of the host larva. (Fig. 5.) It increases rapidly in size as it feeds, and when full grown is from one-half to two-thirds the size of its former host, averaging about 5 to 6 mm in length.

FORMATION OF THE PARASITE COCOON

Shortly after the full-grown *Macrocentrus* larva has finished feeding, construction of the parasite cocoon begins. This cocoon is constructed within the old cocoon of the former host. The shriveled skin, sclerotized head, and thoracic and anal shields of the host larva also remain within the host cocoon. At first a web of very fine, white, silky threads appears about the parasite larva. (Fig. 6, A.) As construction continues the cocoon gradually becomes light brown in color. The completed cocoon (fig. 6, B), which makes a very strong protection about the prepupa and pupa, is dark amber in color and oval in shape and averages from 5.5 to 6.5 mm in length by 1.5 to 2 mm in width. About 24 hours are required for the spinning of the cocoon. The adult emerges from the cocoon by making a slit which opens the round, somewhat thickened anterior end. (Fig. 7.)



FIGURE 6.—Cocoon of *Macrocentrus ancyliorinus*: A, Full-grown larva beginning to construct cocoon; B, completed cocoon shown within a cocoon of the oriental fruit moth. $\times 4.5$

PREPUPAL AND PUPAL PERIODS

The time required from formation of the parasite cocoon to emergence of the adult, including the prepupal and pupal periods, averaged 15.2 days for transforming parasites (Table 2), with a minimum of 9 days and a maximum of 69 days. For wintering parasites, the period of time within the parasite cocoon averaged considerably longer, 38.5 days, with a minimum of 30 days and a maximum of 52 days. This was due to the fact that the cocoons of hibernating parasites were all spun early in the spring when the weather was cool, and thus the prepupal and pupal periods were of longer duration.

The time spent within the cocoon by unparasitized *Grapholitha* has been separated into two periods, prepupal and pupal. (Table 2.) The former period, indicated as "formation of cocoon to pupation" averaged 3.9 days for transforming moths and 202.8 days for wintering moths. The remainder of the time within the cocoon, "pupation to emergence of adult," required an average of 10.6 days for transforming *Grapholitha* and 32 days for wintering ones, the minimum and maximum time for this period being, respectively, 7 and 15 days for transforming individuals, and 9 and 40 days for wintering individuals.

LIFE CYCLE OF PARASITE AND HOST

The life cycle of *Macrocentrus ancyliivorus*, from deposition of the egg to emergence of the adult, averaged 32.1 days for the 600 transforming parasites and 247.6 days for the 49 wintering parasites in observation vials during the two seasons. Records on the life cycle were also available from parasites the host cocoons of which had been spun in corrugated paper. When the two sets of records were combined, the life cycles of the 1,235 transforming parasites were found to average 31.2 days, the minimum and maximum life cycles being 20 days and 91 days, respectively. The minimum, average, and maximum life cycles of the 327 wintering parasites in the combined records were 216, 243.3, and 280 days, respectively.

For unparasitized fruit moths reared in observation vials during the two seasons the average life cycles were 39.2 days for 238 transforming moths and 269.7 days for 94 wintering moths. When records of those individuals that had spun cocoons in corrugated paper were included, the minimum, average, and maximum life cycles of 652 transforming moths were 23, 36.3, and 62 days, respectively, and the corresponding life cycles of 337 wintering moths were 236, 274.3, and 337 days, respectively.

The life cycle of the parasite is in each instance considerably shorter than that of the host species. The average life cycle of all transforming parasites was 5.1 days shorter than that of all transforming moths, and for all wintering parasites 31 days shorter than for all wintering moths.

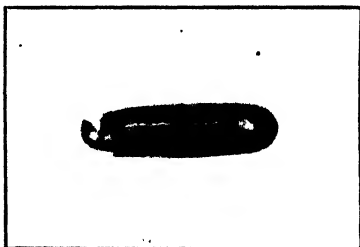


FIGURE 7.—*Macrocentrus* cocoon from which the adult has emerged, showing opening at the anterior end. $\times 4.5$

AVERAGE LIFE CYCLE AND RELATION TO TEMPERATURE

The average number of days required for the life cycle of transforming parasites and moths maturing from eggs deposited on each day throughout the seasons of 1927 and 1928 is shown in Figures 8 and 9. The average life cycles of all transforming *Macrocentrus* that emerged as adults throughout the season are indicated by the height of the solid day line above the base line on the date of oviposition. The average life cycles of transforming *Grapholitha* that emerged as adults throughout the season are similarly shown by the height of the dotted day line above the base line on each oviposition date.

The relation between prevailing temperature and the length of the life cycle in each case is also shown. The height of the solid and dotted temperature lines for each date indicates the average of all temperatures which existed during the average life-cycle periods of all individuals that emerged as adults from eggs deposited on that date, for parasites and moths, respectively. For example, the average life cycle of all transforming *Macrocentrus*, the eggs of which were deposited on June 27, 1927, was 26 days; and the average temperature recorded for that period, as indicated by the solid temperature line on that date, was 72.8° F. This is the average of all temperatures for 26 days, starting with June 27, based on thermograph readings at 2-hour intervals throughout the entire period. The average life cycle of all transforming *Grapholitha*, the eggs of which were deposited on that same date, was 32.3 days; and the average temperature recorded for that period, as indicated by the dotted temperature line on June 27, was 73.2° F. This is the average of all temperatures for 32.3 days starting with June 27.

SPRING EMERGENCE OF HIBERNATING PARASITES AND MOTHS

It has previously been pointed out by the writer (3) that, in the vicinity of Riverton, N. J., adults of *Macrocentrus ancylovorus* emerge sufficiently early in the spring under field conditions to attack the first fruit-moth larvae which enter twigs.

During the winter of 1927-28 *Grapholitha* larvae which had been subjected to parasitism by *Macrocentrus* in the insectary during the fall of 1927 were allowed to hibernate outdoors exposed to natural conditions of temperature and weather. The larvae had constructed cocoons in narrow strips of corrugated straw paper which were firmly tacked in open screen cages, identical with one of the types of cages (fig. 10) employed by Peterson and Haeussler (5) in conducting spring-brood emergence studies of the oriental fruit moth. Two cages, each containing approximately 230 cocoons, were hung on a post. One cage was placed on the south side of the post, as close to the ground as possible, and the other was placed on the north side of the post, about 5 feet above the ground. Previous experiments with hibernating oriental fruit moths and codling moths had shown that the larvae in cages placed in these two positions were subjected to the two extremes of temperature and sunlight during the spring to which larvae normally hibernating on the trunks of trees in the orchard are exposed, and that, consequently, the earliest and latest emergence of adults in the spring occurs in the south-low and north-high cages, respectively.

As had been expected, the first emergence of parasites in the spring of 1928 occurred in the south-low cage, and, as a matter of fact, all

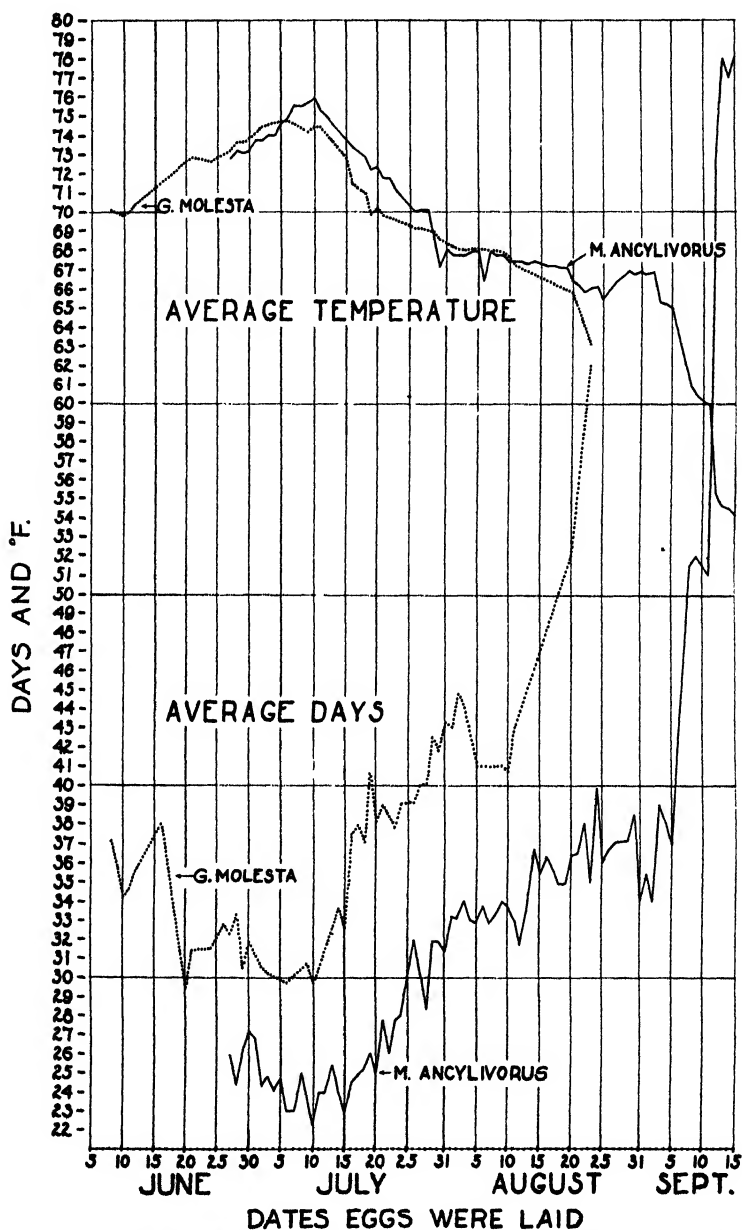


FIGURE 8.—Comparative average life cycles of transforming individuals of *Macrocentrus ancylivorus* (solid line) and *Grapholitha molesta* (dotted line) with corresponding average temperatures in 1927. The life cycle is indicated by the height above the base line (at 0, not shown) on the date on which the eggs were laid. The temperature indicated for that date is the average temperature for the entire life cycle that began on that date

the parasites in this cage had emerged two days before emergence began in the north-high cage. The first adult *Macrocentrus* appeared in the south-low cage on May 11, the peak of emergence (that is, the date on which 50 per cent had emerged) occurred on May 17, and the last parasite emerged on May 19. In the north-high cage, first emer-

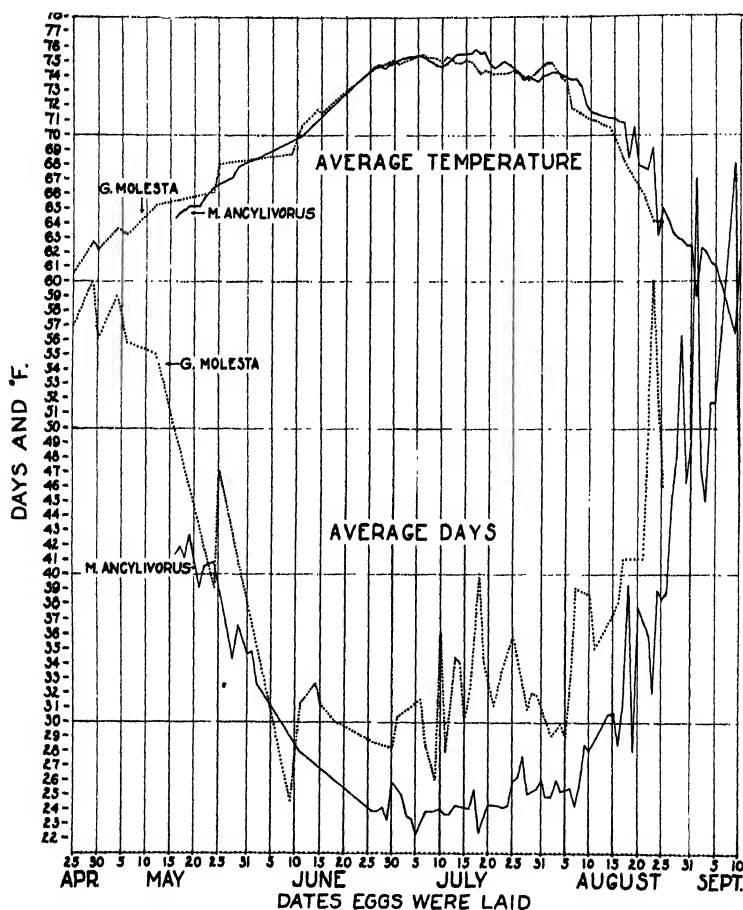


FIGURE 9.—Comparative average life cycles of transforming individuals of *Macrocentrus ancylivorus* (solid line) and *Graphoitha molesta* (dotted line) with corresponding average temperatures in 1928. The life cycles and temperatures are indicated as explained in the legend of Figure 8 and in the text

gence was on May 21, the peak of emergence was reached on May 24, and the last emergence occurred on June 12.

Two similar cages containing unparasitized larvae of the host species had been placed in corresponding positions on a near-by pole. The first adult moth emerged in the south-low cage on April 8, the peak of emergence in this cage was reached on April 30, and last emergence occurred on May 7. In the north-high cage the first moth

emerged on May 8, the peak of emergence occurred on May 16, and the last moth emerged on June 12.

By combining the emergence data from the south-low and north-high cages, in the case of both the parasite and the moth, a general idea is obtained of the spring emergence of the two insects closely approximating that which normally occurs in the peach orchards, assuming that hibernating cocoons under natural conditions are more or less evenly distributed in these two extreme and all intermediate locations.⁵

The comparative emergence of the parasite and the host species in the outdoor cages and in the insectary during the spring of 1928 is shown in Figure 11. The solid lines represent parasite emergence and the broken lines emergence of the moth. The dates of first and last emergence are indicated on the base line and the peak of the triangle denotes the date on which the peak of emergence was reached in each instance.

It will be noted that in the outdoor cages the first moth emerged on April 8, more than a month earlier than the emergence of the first parasite on May 11. Of both species, the earliest individuals to emerge were males, the first female moth emerging on April 20, which was 25 days before the emergence of the first female parasite on May 15. The peak of moth emergence was reached on May 4, which was 19 days before the peak of parasite emergence on May 23. The last individuals of both parasite and host species emerged on June 12.

Spring emergence was considerably delayed in the case of hibernating cocoons which had been kept in the insectary throughout the winter and spring. As shown in Figure 11, the first moth from this material emerged on May 10, the peak of emergence was reached on May 30, and the last moth emerged on July 1. This represents a delay of 32, 26, and 19 days, respectively, as compared to moth



FIGURE 10.—Type of outdoor hibernation cage from which the spring-emergence records were obtained for both parasites (*Macrocentrus ancyliorvus*) and moths (*Grapholitha molesta*)

⁵ A closer approximation of normal orchard emergence would undoubtedly have been obtained by the use of eight cages, four adjacent to the ground and four 5 feet above the ground, with one cage of each group facing north, east, south, and west. Data regarding the emergence of the host species were available for the entire eight cages but since sufficient hibernating parasite material was available for only two cages, the emergence data for the moths, as given here, were taken from only the two corresponding cages of hibernating *Grapholitha* material.

emergence in the outdoor cages. In the case of the parasites the delay was not so great. Nevertheless a retardation of emergence occurred, the first adult (a female) appearing on May 19, 8 days later than the first parasite emerged in the outdoor cages. The peak of emergence was delayed 4 days, being reached on May 27; and the last parasite emerged on June 13, only 1 day after the last individual emerged in the outdoor cages. Thus it is apparent that, in the case of the parasites as well as the moths, a closer approximation to the normal spring emergence can be obtained by the use of outdoor hibernation cages. In the insectary, the first parasite emerged 9 days later than the first moth. The peak of parasite emergence, however, was reached 3 days in advance of the peak of moth emergence, and the last moth emerged 18 days later than the last parasite.

SEASONAL EGG DEPOSITION

A comparison of the periods during which fruit-moth eggs and parasite eggs were deposited in 1927 and 1928, from which transforming and wintering individuals of *Grapholitha* and *Macrocentrus*

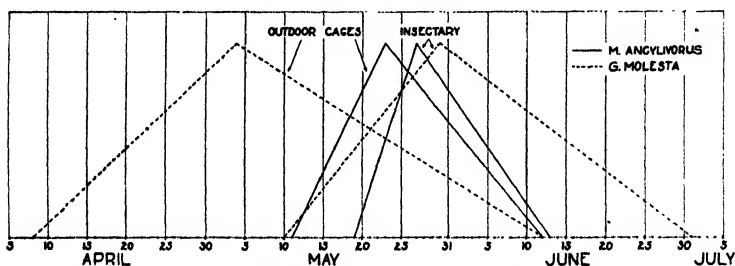


FIGURE 11.—Comparative emergence of adults of *Macrocentrus ancylivorus* and *Grapholitha molesta* during the spring of 1928 from larvae that hibernated in outdoor cages and from larvae that hibernated in the insectary

subsequently emerged, is presented in Figure 12. The dates on which the first and last eggs producing transforming and wintering individuals were deposited are indicated in each instance by the range of the horizontal lines, the broken and solid lines representing the fruit-moth eggs and parasite eggs, respectively.

In 1927, the first *Grapholitha* eggs of the season were deposited in the insectary on April 23 by females which emerged in outdoor cages, but the first eggs on which developmental records were kept in connection with the parasite studies were deposited on June 8. The last eggs that produced transforming moths were deposited on August 23. The first eggs producing wintering *Grapholitha* were deposited on July 27. Thus both transforming and wintering fruit moths developed from eggs deposited between July 27 and August 23, inclusive. The last eggs that produced wintering *Grapholitha*, which were also the last eggs of this species laid that season, were deposited on September 24.

Since it was not possible to begin the parasite life-history studies in 1927 until adults of the first brood began to emerge from parasitized host larvae collected in the field, the exact date on which the first parasite eggs of that season were deposited is not known. However, the first *Grapholitha* larvae found in peach twigs in the orchard that

season, which were found on May 11, were parasitized by *Macrocentrus ancyliorvus*. Therefore, it is certain that female parasites had emerged from hibernating material and deposited eggs in the field by that date. The parasite studies in the insectary, which were started with the deposition of the first eggs of the second brood on June 27, showed that the last parasite eggs that produced transforming parasites were deposited on September 15. The first eggs that produced wintering parasites were deposited on August 21. Thus, parasite eggs deposited between August 21 and September 15, inclusive, produced both transforming and wintering *Macrocentrus*. The last parasite eggs of the season, the progeny of which survived hibernation and emerged as adults the following spring, were deposited on November 2.

In 1928 it was possible to begin the studies in the insectary with both moths and parasites that emerged from hibernation in outdoor cages. The first *Grapholitha* eggs were deposited on April 25, and

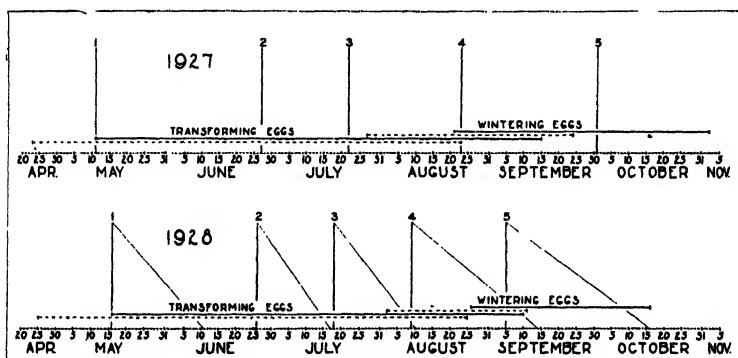


FIGURE 12.—Comparative periods during which eggs were deposited by *Macrocentrus ancyliorvus* (solid line) and *Grapholitha molesta* (dotted line) and number of generations of *M. ancyliorvus* in 1927 and 1928. The period of egg deposition for each generation of parasites in 1928 is indicated by the slanting line which reaches the base on the date the last egg was laid. The beginning of each generation is marked by a vertical line with the number of the generation at the top

the last eggs from which transforming fruit moths developed were deposited on August 25. Eggs producing wintering *Grapholitha* were deposited from August 2 to September 11, inclusive. Both transforming and wintering *Grapholitha* developed from eggs deposited from August 2 to August 25, inclusive.

Eggs that produced transforming parasites in 1928 were deposited from May 16 to September 10, inclusive, and eggs producing wintering parasites were deposited from August 26 to October 16, inclusive. Both transforming and wintering *Macrocentrus* developed from parasite eggs deposited between August 26 and September 10, inclusive.

NUMBER OF GENERATIONS

Five generations of *Macrocentrus ancyliorvus* occurred during each of the seasons of 1927 and 1928. The dates on which the first eggs of each brood were deposited during each season are indicated in Figure 12. The period of egg deposition for each brood in 1928 is also shown, being indicated by the slanting line terminating at the

date on which the last egg was laid. Similar records were not available for 1927.

In 1927 the first and second broods were entirely transforming. All of the third-brood eggs, except a few of the latest ones deposited, produced transforming parasites. About two-thirds of the eggs of the fourth brood produced transforming individuals, and the remainder produced wintering ones. The fifth brood was entirely a wintering one. There were only four generations of the host species during that season.

In 1928 the first three broods of parasites were entirely transforming. Brood 4 contained a decided majority of transforming individuals, and brood 5, except for a few from the very earliest eggs that were deposited, produced wintering individuals. A period of about two weeks elapsed between the time of deposition of the last eggs of the first brood and the first eggs of the second brood in 1928. This was due to the fact that practically all the female parasites which emerged in the spring from hibernating material had died by the middle of June, and as an average life cycle of 41.3 days was required for development of the earliest individuals of the first brood, eggs of which were deposited on May 16, first-brood adults did not begin to emerge until about June 26. Thus, female parasites were not present in the insectary to deposit eggs from June 11 to June 26. A decided decrease in parasitism by *Macrocentrus ancyliivorus* also occurred in the field during this same period in 1928, as indicated by weekly collections of *Grapholitha* larvae from infested peach twigs. These data have been presented in tabular form by the writer (3, p. 373). Parasitism by this species, which had reached a peak of 49.9 per cent in the field at the collection of May 29-June 1, gradually decreased to a minimum of 17.9 per cent for the period June 19-20. It then increased to 29.5 per cent for the period June 26-28, and the following week, July 3-5, it jumped to the high peak of the season, 67.9 per cent. Since the twigs were heavily infested by larvae of the host species throughout this entire period it is evident that the decrease in parasitism in the field was also due to a scarcity of female parasites during the two weeks immediately preceding the emergence of first-brood adults late in June. Five complete or partial generations of the host species occurred in 1928.

HIBERNATION

Macrocentrus ancyliivorus hibernates as a first-instar larva within the body of the full-grown hibernating host larva. In the spring of 1929, a few weeks before wintering parasite larvae would begin to emerge from the host larvae, a number of hibernating larvae of the oriental fruit moth that had been parasitized by *M. ancyliivorus* the previous fall were dissected. Each *Grapholitha* larva contained one live first-instar parasite larva and, in most instances, also several head capsules and bodies of dead parasite larvae. Measurements of the head capsules of the dead parasite larvae showed them to be of the same instar as the live ones.

During both 1927 and 1928 a considerable number of parasite larvae reached the cocoon stage late in the fall but failed to emerge as adults. Such cocoons were allowed to go through the winter undisturbed in order to ascertain whether any individuals might success-

fully hibernate within the parasite cocoon. In no instance did adults emerge in the spring from parasite cocoons spun the previous fall. The majority of these cocoons, when opened, were found to contain dead pupae, which suggests that these individuals pupated late in the fall but were unable to survive the sudden drops in temperature which occurred before they could emerge.

Considerable mortality after the parasite cocoon stage was reached also occurred in parasites which survived hibernation within the host and reached the cocoon stage in the spring. In 1928 and 1929 50 and 63 per cent, respectively, of the hibernating parasites that emerged from the host and spun cocoons in the spring failed to emerge as adults. Subsequent examination of these did not reveal the cause of death, although it was found that the majority had died as prepupae.

SUMMARY

Macrocentrus ancyliivorus Roh. is an important parasite of the oriental fruit moth in the vicinity of Riverton, N. J. A closely related species, *M. delicatus* Cress., is parasitic on the same host species, but adults of *ancyliivorus* and *delicatus* have distinguishing characters by which living specimens may be separated.

A greater percentage of female than of male parasites is produced when larvae of the oriental fruit moth and larvae of the strawberry leaf roller are parasitized by *Macrocentrus ancyliivorus* in the field. A consistently greater proportion of females does not always occur when parasitism takes place under insectary conditions.

The average longevity of adult parasites of both sexes confined together in cages is 6.7 days; the average for unfertilized females confined alone is 17.9 days, and for males confined alone, 10.3 days.

Oviposition is possible only when the larva of the host species is in a position in which it can not escape the ovipositor of the female parasite. Superparasitism occurs, but only one parasite larva survives.

Unfertilized females produce only males.

Fruit-moth larvae of all sizes are subject to attack by this parasite. Parasitism in the field occurs chiefly in larvae of the early broods which feed in peach twigs.

As many as 40 offspring have been produced by a single female of *Macrocentrus ancyliivorus* under insectary conditions.

The transforming parasite larva emerges from the host, on an average, 3 days after the *Grapholitha* larva would normally have pupated, and feeds on it for 12 to 18 hours. Wintering parasite larvae emerge from the host larvae in the spring and require an average of 2 days for feeding. About 24 hours are required for formation of the parasite cocoon. The combined prepupal and pupal period averages 15.2 days for transforming parasites and 38.5 days for wintering parasites. The average life cycle of transforming and wintering *Macrocentrus* is, respectively, 5.1 and 31 days shorter than the average life cycle of transforming and wintering *Grapholitha*.

Both adult parasites and moths emerge earlier in the spring from larvae which hibernate in outdoor cages than from larvae which hibernate in the insectary. In outdoor cages the spring emergence of the moths occurs considerably earlier than the spring emergence of the parasites.

During late summer and early fall there is a period during which eggs deposited by *Macrocentrus* and by *Grapholitha* may produce both transforming and wintering progeny.

There were five generations of this parasite in the Riverton-Moorestown area in 1927 and in 1928.

Macrocentrus hibernates as a first-stage larva within the full-grown hibernating larva of the oriental fruit moth. Many parasite cocoons that are constructed late in the fall and in the spring fail to produce adult parasites owing to mortality of the prepupae and pupae.

LITERATURE CITED

- (1) DRIGGERS, B. F., and PEPPER, B. B.
1931. *MACROCENTRUS ANCYLIVORA* ROHR. AND *M. DELICATUS* CRESS. DISTINCT SPECIES. *Ann. Ent. Soc. Amer.* 24:293-301, illus.
- (2) FINK, D. E.
1926. THE BIOLOGY OF *MACROCENTRUS ANCYLIVORA* ROHWER, AN IMPORTANT PARASITE OF THE STRAWBERRY LEAF ROLLER (*ANCYLIS COMPTANA* FROEHL.). *Jour. Agr. Research* 32:1121-1134, illus.
- (3) HAEUSSLER, G. J.
1930. PARASITES OF THE ORIENTAL PEACH MOTH, *LASPEYRESIA MOLESTA* BUSCK, IN NORTH AMERICA. *Jour. Agr. Research* 41:365-377, illus.
- (4) PETERSON, A., and HAEUSSLER, G. J.
1926. THE ORIENTAL PEACH MOTH. U. S. Dept. Agr. Circ. 395, 28 p., illus.
- (5) ——— and HAEUSSLER, G. J.
1928. DETERMINATION OF THE SPRING-BROOD EMERGENCE OF ORIENTAL PEACH MOTHS AND CODLING MOTHS BY VARIOUS METHODS. *Jour. Agr. Research* 37:399-417, illus.
- (6) ——— and HAEUSSLER, G. J.
1930. LIFE HISTORY OF THE ORIENTAL PEACH MOTH AT RIVERTON, N. J., IN RELATION TO TEMPERATURE. U. S. Dept. Agr. Tech. Bul. 183, 38 p., illus.
- (7) ROHWER, S. A.
1923. A NEW *MACROCENTRUS* REARED FROM THE STRAWBERRY LEAF-ROLLER (HYMENOPTERA, BRACONIDAE). *Ent. Soc. Wash. Proc.* 25:168.
- (8) STEARNS, L. A.
1928. THE LARVAL PARASITES OF THE ORIENTAL PEACH MOTH (*LASPEYRESIA MOLESTA* BUSCK) WITH SPECIAL REFERENCE TO THE BIOLOGY OF *MACROCENTRUS ANCYLIVORA* ROHWER. N. J. Agr. Expt. Sta. Bul. 460, 24 p., illus.

EFFECT OF SIZE OF CROWN AND LENGTH OF CUTTING SEASON ON YIELDS OF ASPARAGUS¹

By E. S. HABER

Assistant Chief in Vegetable Crops, Iowa Agricultural Experiment Station

INTRODUCTION

Plants of *Asparagus officinalis* L. produced from field-run seed vary potentially from very poor yielding to very good yielding, the largest number being intermediate in yield. Nurserymen selling asparagus crowns usually grow them from seed planted in a nursery row. The seedlings are allowed to remain in the row for one or two years before they are transplanted to the permanent plantation. Many 1-year-old crowns are small, especially if the seed is sown too thick. These small roots may have genetic potentialities for larger yields. Should they be discarded or will they produce satisfactory yields?

Root selection in asparagus has been advocated for a number of years. In selecting for size, however, there is always the possibility that crowns discarded because of their smallness might have produced satisfactory yields had they been planted in the permanent location. The small size might have been due entirely to environmental conditions in the nursery row, such as crowding, poor soil, or lack of moisture, and not to factors of heredity. Under favorable conditions for growth the plant might have reached normal size and, since cutting is usually delayed for two years after the crowns are planted, the small crowns might have yielded as well as the large ones.

The purpose of the investigation herein reported was to determine (1) the effect of size of root at planting on the subsequent yield of the plant, and (2) the effect of length of cutting season on yields.

REVIEW OF LITERATURE

Robbins and Jones (6, 7)² found that staminate plants outyield pistillate plants. This difference in yield is greater early in the season than later, and the pistillate spears are, on an average, larger than the staminate spears. This confirms the findings of Green (2) and Tiedjens (9).

Myers (5) reports the yields of three grades of crowns for a period of six years. He found little difference in yield between first and second grade crowns, but small crowns produced yields decidedly inferior to the first two. Jones and Robbins (4) found that desiccated crowns produced 12.4 per cent less the first year and 27 per cent less the second year than nondesiccated crowns. Unpruned crowns outyielded pruned crowns by 14 per cent the second season. The same authors (3) concluded that no injury resulted from harvesting spears for a short time the year after planting. Schermerhorn (8) concluded that the diameter of brush did not necessarily indicate the diameter

¹ Received for publication Dec. 14, 1931; issued August, 1932. Journal Paper No. B 22 of the Iowa Agricultural Experiment Station.

² Reference is made by number (italic) to Literature Cited, p. 109.

of the spears that might be cut the following spring. A more accurate index of plant performance is the actual diameter and number of spears per plant.

RELATION OF SIZE OF CROWN TO YIELD

In 1927 an experiment was started to determine what effect the size of the roots at planting time would have on the yield of the plants. One hundred and fifty 1-year-old crowns were dug from the nursery row in the spring before growth started. These were taken to the laboratory immediately, washed free of earth, surface-dried with a soft cloth, weighed, and immediately planted in the field.

The roots were not selected but were taken at random from the nursery row. They ranged in weight from 3 to 57 grams. They were planted 4 by 2 feet in 1927, and no cuttings were made until the spring of 1929. When the plants bloomed, it was found that there were 79 male and 71 female crowns. The records of cuttings were kept for each plant, the total weight of spears and the number of spears produced at each cutting being recorded. The spears were cut when 6 to 8 inches high. Cuttings were made until June 15 each year.

Unless individual yield records have been kept for asparagus plants, it may be thought that an asparagus plant yields continuously at every cutting throughout the season. This is not the case with every plant, and very few plants yield marketable spears at every cutting. Table 1 gives the individual yield records of some high and some low yielding plants for 1931. It may be seen from this table that many plants yield very little throughout the season, while others are comparatively heavy yielders. The roots of the heavy-yielding plants averaged 20 grams each when planted and those of the low-yielding plants averaged 16 grams each.

In Table 2 are presented the number of spears, total weight of spears, and average weight of spears for each sex for the first three cutting seasons.

Male crowns produce a greater number of spears and a heavier yield per plant than female crowns. On the other hand, female crowns produce spears of larger diameter, as is indicated by the average weight of spears. The average weight of the male crowns at planting was slightly greater than that of the female crowns. However, if all crowns that weighed 15 g or less had been discarded, more male crowns would have been discarded than female crowns. These observations are only incidental to the main problem and are in harmony with the results reported by other investigators.

The question arises as to the advisability of planting all male plants rather than both male and female. According to Robbins and Jones (6), asparagus seedlings in California frequently bloom in September of the year in which the seed is sown, so that it is possible to select 1-year-old male plants, but in the Northern States plants do not bloom the first year and a few do not bloom the second year. One-year-old crowns are superior to 2-year-old crowns, so that it would not pay, probably, to grow them two years to determine the sex.

TABLE 1.—*Yields of certain low and high producing asparagus plants, on various dates in 1931*

[The figure before the dash represents the number of spears, and the figure after the dash, the weight of the spears in grams]

Plant No.	Apr. 18	Apr. 24	May 2	May 5	May 15	May 19	May 23	May 25	May 27
15	3-60	1-8	2-58	1-24	3-56	3-62	3-62	1-40	
28		3-20	8-118	4-96	5-120	3-52	2-18	5-96	3-28
46		8-90	2-16	5-42	5-40	6-90	3-90	1-10	7-62
102		3-126	2-64	4-112	4-232	1-36	2-80	2-54	
106	6-46	1-8	9-84	3-44	10-296		3-82	1-18	4-32
118	1-14		8-102	4-80	2-42	6-180	2-20	3-52	2-26
31					1-16				
44		1-20	1-8	1-10		1-12			
61							1-30		
119				1-24		1-24			
120						1-26			
115				1-32	1-18	2-66			1-26
89				1-08	1-52	1-22			2-112
92					1-18				

Plant No.	May 29	June 1	June 4	June 6	June 9	June 11	June 13	Total number of spears	Total weight of spears
15		1-8	2-36	2-48	4-54	1-18		27	534
28	2-26		9-258	1-10	5-72	2-16	4-64	56	994
46		3-32	1-8	6-112		4-42	6-56	57	680
102	4-106		2-32	1-24		3-62	2-52	30	960
106	4-66	2-62	1-16	6-94		5-48		55	896
118	1-16	3-48	5-130	3-58	3-38	1-16	2-16	46	838
31			1-18			1-24		3	58
44				1-8		1-12		6	70
61		1-26				1-6	1-20	4	82
119				1-18				3	66
120		1-26		1-20				3	72
115		1-28		2-106				8	276
89		1-18				1-76		7	348
92		1-14						2	32

TABLE 2.—*Yields of male and female asparagus plants, 1929-1931*

[The standard deviation rather than the probable error follows each mean]

Plants	Average weight of crowns when planted	1929		
		Average weight of spears	Spears per plant	Total weight per plant
Male.....	Grams 22.11±1.74	Grams 17.35±0.57	Number 9.59±0.52	Grams 162.83±8.59
Female.....	Grams 20.46±1.87	Grams 17.69±.43	Number 7.02±.35	Grams 119.78±5.90
Average.....	Grams 21.08±1.78	Grams 17.49±.56	Number 8.48±.47	Grams 144.25±7.36

Plants	1930			1931		
	Average weight of spears	Spears per plant	Total weight per plant	Average weight of spears	Spears per plant	Total weight per plant
Male.....	Grams 16.82±0.55	Number 15.37±0.88	Grams 246.68±12.28	Grams 18.74±0.15	Number 21.33±0.97	Grams 393.35±3.92
Female.....	Grams 21.09±.51	Number 9.39±.05	Grams 184.44±8.91	Grams 21.56±.05	Number 11.05±.58	Grams 248.17±2.95
Average.....	Grams 18.66±.53	Number 12.79±.73	Grams 219.82±10.96	Grams 19.96±.09	Number 16.89±.78	Grams 332.37±3.53

On the top line, or line A, of Table 3, are presented the correlation coefficients between weight of crown at planting and average weight of spears; weight of crown and number of spears per plant; and weight of crown and total weight per plant for the years 1929, 1930, and 1931. The correlation coefficients were calculated separately for the male and female plants and the total correlation coefficients presented in Table 3 were secured by pooling according to Fisher's (1) method, which gives the same benefit as the use of a larger number of observations.

In lines B, C, D, etc., Table 3, are presented the correlation coefficients between average weight, total weight, and number of spears for three years. These were obtained as a matter of interest and have no bearing on the coefficients between crown weight and other variables presented in line A. The tests of significance were taken from Wallace and Snedecor's (10) table of significant values of r , R , and t adapted from Fisher's (1), tables of t and z .

TABLE 3.—Correlation coefficients for asparagus Plants, 1929–1931

MALE PLANTS									
A	B	C	D	E	F	G	H	I	J
Weight of crown	Average weight of spears, 1931	Number spears per plant, 1931	Total weight per plant, 1931	Average weight of spears, 1930	Number spears per plant, 1930	Total weight per plant, 1930	Average weight of spears, 1929	Number spears per plant, 1929	Total weight per plant, 1929
A.....	0.0135	* 0.2745	* 0.2521	0.0439	* 0.2699	* 0.3101	0.0659	* 0.3691	* 0.3447
B.....		-.0816	* .2771	* .6148	-.0639	.2414	* .4135	.1587	.2469
C.....			* .8874	-.1195	* .9016	* .7779	-.0146	* .7113	* .6263
D.....				.0889	* .7945	* .8472	.1317	* .7657	* .7556
E.....					-.1843	* .3043	* .6162	-.0329	* .2641
F.....						* .8259	-.0882	* .7029	* .5464
G.....							.2158	* .7166	* .7430
H.....								-.0270	* .3700
I.....									* .8736
FEMALE PLANTS									
A.....	0.0167	-.0.1808	-.0.1725	-.0.0275	* -.0.3024	* -.0.3281	0.0201	-.0.1848	-.0.1771
B.....		-.0566	* .3648	* .5388	-.0550	* .3279	* .5858	-.1167	.1503
C.....			* .7255	-.1945	* .7475	* .6639	-.1226	* .7785	* .6008
D.....				.1001	* .6106	* .7582	.0540	* .6007	* .5279
E.....					* -.3134	.2378	* .6031	-.1342	.1991
F.....						* .8047	-.2002	* .7567	* .5518
G.....							.1623	* .6510	* .6465
H.....								.1294	* .4387
I.....									* .8075
ALL PLANTS									
A.....	0.0147	0.1603	0.1139	0.0082	0.1241	0.0074	0.0465	0.1818	0.1577
B.....		-.0622	* .2919	* .5678	-.0531	* .2717	* .4986	.0331	* .2301
C.....			* .8453	-.1233	* .8787	* .7259	-.0388	* .7092	* .6024
D.....				.0878	* .7521	* .8172	.1025	* .7217	* .6889
E.....					* -.2039	* .2640	* .6026	-.0726	* .2250
F.....						* .8017	-.0114	* .7099	* .5378
G.....							.1934	* .6944	* .7107
H.....								.0029	* .3927
I.....									* .8530

* Significant.

* Highly significant.

The average weight of spears was not correlated with size of crown in male or female plants in any of the three years' data recorded. Total yield or total weight of spears was significantly correlated with weight of crown in male plants for three years; in 1929 the correlation

coefficient was highly significant. With the female plants there was a negative correlation between yield and weight of crown, but in 1930 only was this negative correlation significant, and then only slightly so. The correlation coefficients between number of spears per plant and weight of crown were slightly significant in male plants, but only one significant negative correlation was found in female plants.

There are no significant correlations between average weight of spears and number of spears in either sex except in the female plants in 1930, when a slightly significant correlation was found. Total weight of spears per plant and number of spears are highly correlated in the same year, or what one might expect the following year or two years later.

Total weight of spears in any one year is highly correlated with total weight of spears in each of the other two years, and the same relationship holds true for number of spears and average weight of spears for either sex; i. e., if one secures a high yield or a large number of spears, or spears of large diameter from crowns for one season, one may expect approximately the same relationship in following years.

EFFECT OF THE LENGTH OF THE CUTTING SEASON ON YIELDS

A matter of interest to professional as well as to amateur gardeners is the season of year and the length of time asparagus may be cut without detriment to future yields. Since there is a ready sale for the product quite late in the summer, this matter is important.

To determine the effect of length of cutting season on yields, a series of plots was planted in 1927. The plants were set 2 feet apart in rows 4 feet apart and 100 feet long. The soil was Webster silt loam, and no fertilizers were applied either before or after planting. The first cuttings were made in 1929. Three years' records, 1929, 1930, and 1931, are now available. Each year rows were cut as follows: 6 rows were cut up to and including May 1; 8 rows were cut up to and including May 15; 8 rows were cut up to and including June 1; 8 rows were cut up to and including June 15; 6 rows were cut up to and including July 1; and 6 rows were cut up to and including July 15. The rows to be cut were selected at random at the beginning of the experiment. They were not located in a solid block but were scattered through the field.

In Table 4 are presented the average total yield and the average number of spears for the replicates in each series.

TABLE 4.—Average yield and average number of spears of the asparagus replicates for each date of cutting

Date when cutting ceased	Weight of asparagus cut	Spears cut	Date when cutting ceased	Weight of asparagus cut	Spears cut
	Ounces	Number		Ounces	Number
May 1.....	1929 34.3±2.44	79.0±5.58	June 15.....	1929 290.0±14.75	496.9±24.42
	1930 46.8±2.88	55.5±4.10		1930 282.5±13.39	479.7±24.71
	1931 93.0±13.46	150.8±20.10		1931 470.8±25.12	795.3±25.12
May 15.....	1929 83.4±4.32	159.0±7.50	July 1.....	1929 388.8±19.37	672.0±30.50
	1930 174.9±10.32	238.1±11.24		1930 360.8±10.22	678.3±26.81
	1931 191.8±7.64	243.7±11.60		1931 554.3±19.89	975.2±44.03
June 1.....	1929 208.0±4.58	349.7±7.19	July 15.....	1929 413.2±32.60	770.5±42.43
	1930 217.0±5.18	343.5±9.93		1930 393.5±17.75	678.5±29.71
	1931 342.5±5.97	533.0±11.82		1931 408.2±41.45	925.0±54.96

All rows cut in 1931 showed an increase in yield over the preceding year, although this increase was slight for rows cut up to and including July 15. All rows showed an increase in number of spears irrespective of length of cutting season in 1931. A slight decrease in yield irrespective of treatment in 1930 as compared to 1929 occurred in rows cut up to and including June 15, July 1, and July 15. Rows cut until May 1, May 15, and June 15 showed only a very small increase in 1930 as compared to 1929. This small increase may be accounted for by the fact that three very heavy freezes occurred after the beginning of the growing season, and it was impossible to obtain yield records for this period since all spears were killed. Also, the severe drought of 1930 affected the June 15, July 1, and July 15 plots. Rows cut until July 15 in 1931 showed a decidedly smaller yield than rows cut until June 15 or July 1, even though the cutting season extended two weeks and one month longer, which represented 7 and 13 more cuttings, respectively.

In Table 5 the mean differences between yields and number of spears are presented along with the probability (*P*) values secured from Fisher's (*t*) table of *t*. There is a significant difference in yields between rows cut until July 1, 1931, and those cut until June 15, 1931. In other words, the yield was greater by cutting longer, and apparently cutting until July 1 in 1929 and 1930 did not cause a decrease in yield in 1931. On the other hand, rows cut until July 15, 1931, did not yield as much as those cut until June 15, although 13 more cuttings were made. The long cutting season in 1929 and 1930 (to July 15) seriously affected the yielding capacity of the rows in 1931, since the value of *P* is not significant between the mean values of June 15 and July 15.

TABLE 5.—Mean differences (*M. D.*) between yields and number of spears, with probability values (*P*) for asparagus plots cut at different dates in 1931

DIFFERENCE BETWEEN PLOTS CUT UNTIL JUNE 15 AND JULY 1			
Item	M. D.	P	Significance
Yield.....ounces.....	83.5±33.87	0.05-0.02	Significant.
Spears.....number.....	179.9±62.84	.02-.01	Do.
DIFFERENCE BETWEEN PLOTS CUT UNTIL JUNE 15 AND JULY 15			
Yield.....ounces.....	-62.6±45.96	0.2-0.1	Not significant.
Spears.....number.....	129.7±74.91	.2-.1	Do.
DIFFERENCE BETWEEN PLOTS CUT UNTIL JUNE 1 AND JUNE 15			
Yield.....ounces.....	128.3±25.83	(*)	Highly significant.
Spears.....number.....	262.3±44.73	(*)	Do.

* Less than 0.1.

TABLE 6.—*Analysis of variance*

TREATMENT OF YIELDS

Variance	Degrees of freedom ^a	Sum of squares	Mean squares	Standard deviation	Log S. D. ^a
Between classes.....	3	101, 370. 5816	33, 790. 1739	183. 821	5. 2185
Within classes.....	24	37, 383. 256	1, 557. 6356	39. 4069	3. 6815
Total.....	27	138, 753. 8376	5, 139. 031	71. 6870	^b 1. 5370

TREATMENT OF NUMBER OF SPEARS

Between classes.....	3	133, 894. 053	44, 631. 351	211. 2613	5. 3598
Within classes.....	24	103, 563. 439	4, 315. 143	65. 6997	4. 1914
Total.....	27	237, 457. 492	8, 794. 721	93. 780	^b 1. 1684

^a 5 per cent point of the distribution of $Z=0.5508$; 1 per cent point=0.7757.^b Difference.

Do rows cut until June 1 yield as well as those cut until June 15? Perhaps cutting even till the middle of June may have caused some decrease in yield in 1931. However, since the value of P is highly significant, it is evident that the yield was increased by cutting until June 15.

It was thought well to determine what the rows yielded in 1931 when yield records were taken to a definite date; in other words, the yields were determined to June 1 for rows that had been cut a longer period during the three years. The same statistical treatment was used for yields to June 15 for rows cut longer than this period. To determine whether differences in weight and number of spears were due to differences in length of cutting season or whether they were due to random sampling, Fisher's (t) analysis of variance was used in a statistical treatment of the data. (Table 6.)

The values for the 5 per cent and 1 per cent points of the distribution of z (taken from Fisher's (t) table of z) for the number of degrees of freedom involved when compared with the difference of the logarithms of the standard deviations, indicate that the differences obtained probably are due to the treatment rather than to random sampling.

In Table 7 the weight and number of spears to June 15 for rows cut after this date are treated by analysis of variance. The mean differences between June 15 and July 1 rows (cut until June 15, 1931) are not significantly different since the values of P are not significant. Mean differences between June 15 and July 15 rows (cut until June 15, 1931) are significantly different, as are the mean differences between July 1 and July 15 rows (cut until June 15, 1931). From this it may readily be seen that cutting until July 15 is entirely too long for maximum yields in the following years.

In Table 8 the weight and number of spears to June 1 for rows cut after this date are treated as in Table 7. No significant differences were found to June 1, 1931, between rows cut until June 1 and June 15 during the two preceding years, nor were there significant differences between June 1 and July 1 rows in yield and number of spears to June 1, 1931. On the other hand, highly significant differences were obtained between June 1 and July 15 rows cut to June 1, 1931, and also between July 1 and July 15 rows cut to the same date. By taking the yields to a definite date for all rows, a comparison may be made which includes the same number of cuttings for all treatments.

TABLE 7.—Mean differences (M. D.) between yields and number of spears, with probability values (P) for asparagus plots cut at different dates in 1931

DIFFERENCE BETWEEN JULY 1 AND JUNE 15 PLOTS CUT UNTIL JUNE 15

Item	M. D.	P	Significance
Yield.....ounces..	55.3±30.91	0.2-0.1	Not significant.
Spears.....number..	90.0±57.24	.2- .1	Do.

DIFFERENCE BETWEEN JULY 15 AND JUNE 15 CUT UNTIL JUNE 15

Yield.....ounces..	201.5±37.06	(*)	Significant.
Spears.....number..	249.5±59.44	(*)	Do.

DIFFERENCE BETWEEN JULY 15 AND JULY 1 CUT UNTIL JUNE 15

Yield.....ounces..	146.2±28.93	(*)	Significant.
Spears.....number..	159.5±48.99	(*)	Do.

* Less than 0.01.

TABLE 8.—Mean differences (M. D.) between yields and number of spears, with probability values (P) for asparagus plots cut at different dates in 1931

DIFFERENCE BETWEEN JUNE 1 AND JUNE 15 PLOTS CUT UNTIL JUNE 1

Item	M. D.	P	Significance
Yield.....ounces..	15.625±20.0066	0.5-0.4	Not significant.
Spears.....number..	47.875±32.4579	.2- .1	Do.

DIFFERENCE BETWEEN JUNE 1 AND JULY 1 PLOTS CUT UNTIL JUNE 1

Yield.....ounces..	36.667±23.4149	0.2-0.1	Not significant.
Spears.....number..	32.000±42.0680	.5- .4	Do.

DIFFERENCE BETWEEN JUNE 1 AND JULY 15 PLOTS CUT UNTIL JUNE 1

Yield.....ounces..	143.833±28.4288	(*)	Highly significant.
Spears.....number..	148.333±42.4404	(*)	Do.

DIFFERENCE BETWEEN JULY 1 AND JULY 15 PLOTS CUT UNTIL JUNE 1

Yield.....ounces..	107.165±19.0848	(*)	Highly significant.
Spears.....number..	116.333±36.0741	(*)	Do.

* Less than 0.01.

SUMMARY AND CONCLUSIONS

Since it is usually impossible to determine the sex of 1-year-old asparagus plants in the Northern States, and since the weight of female crowns average slightly less than that of male crowns, it might be well to discard very small crowns because of the possibility that by so doing a large number of male crowns would be retained.

The results of the correlation studies herein reported confirm the conclusions of several other workers in showing that male crowns produce a greater number of spears and a heavier yield per plant than female crowns and that female crowns produce spears of larger diameter.

Under the conditions of these experiments significant correlations exist in male asparagus plants between the weight of 1-year-old crowns when planted and the number of spears and total weight of

spears produced during the first three cutting seasons. There were no significant correlations between the weight of the crown at planting and the average weight of the spears produced. Female plants during 1930 showed a significant negative correlation between weight of crown and total number of spears and total yield; however, in the preceding year and in the following year no such correlation existed. When male and female plants are pooled, no significant correlation coefficients are found between these factors.

For the three years' data recorded there seems to be an increase in weight and number of spears for all rows irrespective of length of cutting season, except rows cut until July 15. There is an increase in production for 1931 in each successive series except for rows cut until July 15. Rows cut until July 15 in the third year did not yield as much as rows cut until June 15, although 13 more cuttings were made. Rows cut until July 15 did not yield as much as the rows cut until July 1, although 7 more cuttings were made. Cutting until July 1 and June 15 in 1931 greatly increased the crop produced as compared with rows cut until June 1, 1931.

June 1, June 15, and July 1 rows yielded to June 1, 1931, approximately the same amount; at least no significant differences were noted; as compared with these same series, the July 15 rows cut to June 1, 1931, showed a significant decrease in yield. Under the conditions of this experiment, three years' cutting records demonstrate that July 15 is entirely too long to harvest asparagus. The rows cut until July 1 showed no decrease in yield, but the increases over the rows cut a shorter period of time though significant are not highly so, and future records may disclose that this date is entirely too late to harvest.

LITERATURE CITED

- (1) FISHER, R. A.
1930. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 3, rev. and enl., 283 p., illus. Edinburgh and London.
- (2) GREEN, W. J.
1891. ASPARAGUS. Ohio Agr. Expt. Sta. Bul. ser. 2, v. 3, p. [241]-244.
- (3) JONES, H. A., and ROBBINS, W. W.
[1927]. INFLUENCE OF CUTTING ASPARAGUS THE FIRST YEAR AFTER PLANTING ON PRODUCTION THE FOLLOWING YEAR. Amer. Soc. Hort. Sci. Proc. (1926) 23: 23-25.
- (4) ——— and ROBBINS, W. W.
[1927]. INFLUENCE OF DESICCATION AND ROOT PRUNING ON PERFORMANCE OF ASPARAGUS. Amer. Soc. Hort. Sci. Proc. (1926) 23: 26-28.
- (5) MYERS, C. E.
1918. EXPERIMENTS WITH ASPARAGUS. Penn. Agr. Expt. Sta. Ann. Rpt. 1915-16: 557-578.
- (6) ROBBINS, W. W., and JONES, H. A.
1925. SECONDARY SEX CHARACTERS IN ASPARAGUS OFFICIANALIS L. Hilgardia 1: [183]-202, illus.
- (7) ——— and JONES, H. A.
1929. FURTHER STUDIES OF SEX IN ASPARAGUS. Amer. Soc. Hort. Sci. Proc. (1928) 25: 13-16, illus.
- (8) SCHERMERHORN, L. G.
1929. A SUMMARY OF THE PERFORMANCE RECORDS OF INDIVIDUAL ASPARAGUS PLANTS IN 1928. Amer. Soc. Hort. Sci. Proc. (1928) 25: 35-36.
- (9) TIEDJENS, V. A.
[1925]. SOME PHYSIOLOGICAL ASPECTS OF ASPARAGUS OFFICIANALIS. Amer. Soc. Hort. Sci. Proc. (1924) 21: 129-140.
- (10) WALLACE, H. A., and SNEDECOR, G. W.
1931. CORRELATION AND MACHINE CALCULATION. Rev. by G. W. Snedecor. Iowa Agr. Col. Off. Pub. v. 34, no. 4, 71 p.

THE VALUE OF IODINE FOR LIVESTOCK IN CENTRAL PENNSYLVANIA^{1 2}

By E. B. FORBES, *Director, Institute of Animal Nutrition, Pennsylvania State College*; GEO. M. KARNS, *Senior Industrial Fellow, Mellon Institute of Industrial Research*; S. I. BECHDEL, *Professor of Dairy Production, P. S. WILLIAMS, Associate Professor of Dairy Husbandry, T. B. KEITH, Assistant in Animal Husbandry, E. W. CALLENBACH, Associate Professor of Poultry Husbandry, and R. R. MURPHY, Graduate Assistant in Poultry Husbandry, Pennsylvania State College*

INTRODUCTION

A series of feeding experiments was conducted at the Pennsylvania State College in cooperation between the college and the Mellon Institute of Industrial Research—the latter acting through the multiple industrial fellowship of the Iodine Educational Bureau—for the purpose of studying the value of iodine when fed to farm animals, in a manner supplementary to ordinary rations, under conditions present in central Pennsylvania.

In relation to the iodine content of feeding stuffs and waters, central Pennsylvania is sometimes spoken of as a border-line district, in the sense that the needs of domestic animals for iodine are ordinarily satisfied, while an occasional appearance of goiter or of hairlessness in young animals shows that the usual margin of safety in the supply of iodine is so small that a deficiency sometimes occurs.

The essential relation of iodine to the function of the thyroid gland being thoroughly established, and deficiency of iodine in food or water having been shown to be at least a major cause of disturbance of thyroid function, a special interest attaches to the iodine problem in regions such as central Pennsylvania in which there is no acute, general iodine deficiency, since it is not known that supplementary iodine is of value only in regions in which the iodine content of food-stuffs and waters is so deficient as to manifest itself through the prevalence of goiter and related disorders.

In other words it is at least logically possible that iodine might have a nutritive value in relations other than with the thyroid, and the special object of this study was to throw light on this possibility.

SCOPE OF INVESTIGATION

The scope of the investigation was as follows: (1) A feeding experiment was conducted with 25 Holstein-Friesian cows which were infected with *Brucella abortus*, and which were individually subjected to maximum dosage with iodine in an effort to learn the effects of such treatment on the course of the abortion disease; (2) a feeding experi-

¹ Received for publication Dec. 16, 1931; issued August, 1932.

² The general outline of this project was proposed by E. B. Forbes, who also maintained direct oversight of the experimental work and the preparation of the results for publication. Geo. M. Karns devised and prepared the iodine supplement employed, performed the iodine estimations on feedstuffs and drinking water, and cooperated in directing the experiment, through correspondence and personal visits. S. I. Bechdel and P. S. Williams conducted (1) a feeding experiment on cows infected with *Brucella abortus*, to determine the effect of the maximum dosage of iodine on the course of the disease, and (2) a feeding experiment with calves to determine the value of supplementary iodine for growth. T. B. Keith conducted experiments with swine, and also with lambs fed as matched pairs or in groups, to learn the value of supplementary iodine in their growth. E. W. Callenbach and R. R. Murphy conducted a feeding experiment with chickens to learn the effects of iodine on growth and early egg production.

ment with 42 Holstein-Friesian calves, fed as 21 matched pairs, to determine the value of supplementary iodine for growth; (3) a feeding experiment with 40 swine, fed as 10 lots of 4 individuals each; (4) a feeding experiment with 30 lambs fed as 15 matched pairs, and a feeding experiment with 171 lambs, fed as 6 lots of 28 or 29 individuals each, to learn the value of supplementary iodine in the growth of these animals; and (5) a feeding experiment with 701 chickens, fed in 4 groups of 160 to 187 each, to learn the effects of iodine in the growth and early egg production of this species.

In all, therefore, 1,009 animals were fed in this investigation.

The iodine was administered in nearly the whole of this work in the form of a new preparation—iodized linseed meal. This was elemental iodine so combined with linseed meal as to leave but a trace of free iodine.

In the preparation of this compound a certain quantity of old-process linseed meal is placed in a tumbling mill with a known quantity of iodine. The two are then intimately mixed for about 12 hours, by the end of which time the meal and the iodine have become uniformly mixed and a reaction between the two has begun. The preparation is then stored under such conditions that uncombined iodine will not be lost, and the reaction between the two components is completed within a few days. The meal is then standardized as to iodine content.

This preparation was made several times during the course of the experiments, the several lots being standardized to contain from 4 to about 10 per cent of iodine, and the iodine was fed in this form in all the experiments except during a very small portion of the work with calves.

The results of these experiments therefore apply with certainty only to the particular preparation of iodine which was employed, and the reaction of the animals to dosage with this preparation rendered perfectly clear the fact that their tolerance for iodine in this preparation is much less than for iodine as potassium iodide.

The dosage of iodine employed, expressed in milligrams of iodine per day, per 100 pounds live weight, was as follows: Calves, 20; sheep, 30; swine, 50; chickens, 50.

This dosage, which may properly be designated as therapeutic rather than nutritional, in view of the minuteness of the quantities of iodine involved in the functioning of the thyroid gland, was arrived at after a review of published reports on other feeding experiments in which iodine was administered in quantities greater than those required for the maintenance of thyroid function. It is not known that the liberality of the dosage was in any way prejudicial to the state of nutrition of the experimental subjects.

These experiments were conducted with a certain degree of consideration of the locality involved, since the geographic distribution of iodine is significant in relation to animal nutrition; but it was considered more desirable to feed the animals in a manner representative of prevailing custom in the section than to restrict them to locally grown feeds, since central Pennsylvania does not depend in the main on feeding stuffs produced within this section, and it was not practicable to feed these animals as they are normally fed and at the same time feed them exclusively on locally produced products.

The rations, therefore, were composed not only of local products but also, to a large extent, of feedstuffs purchased in the market and of unknown geographic origin. The experiments were all conducted in dry lots. Had they been conducted on pasture the local influence would have been much more pronounced.

The iodine content of the feeding stuffs employed is given in Table 1. In general the iodine content of this group of products does not vary greatly, but as exceptions to this statement the oyster shell was appreciably richer in iodine than were the feeds of agricultural origin; the poultry mashs were also rich in iodine, probably on account of the iodine content of the cod-liver oil contained; and the grain mixture fed to calves contained very much more iodine than is known to be characteristic of any of the feedstuffs of which they are composed. Unfortunately it was impracticable to determine whether some one of the constituent feeding stuffs was responsible for this surprisingly high iodine value, or whether it was due to some unaccountable contamination. In all cases the basal ration contained iodine in quantities sufficient for the maintenance of thyroid function, as estimated from observations by Marine and Kimball on dogs.³

TABLE 1.—*Iodine content and origin of feeding stuffs and water used in various feeding experiments with different kinds of farm animals*

Subjects of experiments	Feeding stuff	Origin	Iodine, parts per billion*
40 swine	Linseed meal	Market, unknown origin	90.3
	Digester tankage	do.	118
	Corn (maize), grain	do.	55.5
	Oats, grain	do.	104
	Wheat middlings	Local mill	
42 calves	Salt	Scranton, Pa.	130
	Water	Spring Creek	1.8
	Grain mixture	Part local, part market	27,300
	Timothy hay	Kylertown, Pa.	115
	Alfalfa hay	New Jersey	78
	Mixed hay	Kylertown, Pa.	60
	Clover hay	Ohio	61.8
	Skim milk	Local	70.7
	Salt	Scranton, Pa.	130
	Water	College well	1.1
171 lambs	Corn (maize), grain	Market, unknown origin	55.5
	Linseed meal	do.	90.3
	Mixed hay	Local	150
	Rock salt	Scranton, Pa.	165
	Water	College well	1.1
30 lambs	Corn	Market, unknown origin	55.5
	Linseed meal	do.	90.3
	Alfalfa hay	Local	78
	Salt	Scranton, Pa.	130
	Water	Spring Creek	1.8
701 chickens	Mash Nos. 1 and 2	Market, Buffalo, N. Y.	1,040
	Mash Nos. 3 and 4	do.	1,090
	Scratch grains	do.	19.4
	Semisolid buttermilk	Local	46
	Oyster shell	Local market	444
	Grit	do.	44.5
	Water	College well	1.1

* Dry basis, except for waters.

³ MARINE, D., and KIMBALL, O. P. THE PREVENTION OF SIMPLE GOITER IN MAN. A SURVEY OF THE INCIDENCE AND TYPES OF THYROID ENLARGEMENTS IN THE SCHOOLGIRLS OF AKRON (OHIO) FROM THE 5TH TO THE 12TH GRADES, INCLUSIVE—THE PLAN OF PREVENTION PROPOSED. JOUR. Lab. and Clin. Med. 3: [40]–48, illus. 1917.

OBSERVATIONS ON THE EFFECTS OF ADMINISTERING IODINE TO COWS INFECTED WITH BRUCELLA ABORTUS

In the study to determine the reaction of cows affected with abortion disease to heavy dosages of iodine, the 25 cows used as subjects were from a herd of grade Holstein-Friesian cattle, maintained for experimental purposes on college farm No. 12, which is 2½ miles from the main dairy barn of the college.

Regular periodic blood tests revealed that this herd was free from abortion disease until the summer of 1929. At this time an outbreak of infectious abortion occurred, and vigorous steps were at once taken to prevent its spread. All reacting or suspected animals were removed to a quarantine barn about 2 miles away, but in spite of the fact that blood tests were made every two weeks, it seemed impossible to prevent a rapid spread of the disease throughout the herd. In fact 31 of the 40 cows reacted to the blood test before the spread of the disease was finally stopped.

As the plan of management included the holding of the reacting animals in quarantine for some time, an excellent opportunity was afforded for the experimental administration of iodine to infected cows and to some others which had been exposed but which had not reacted to the agglutination blood test.⁴

Iodized linseed meal was given, therefore, with the regular ration, in as large quantities as the animals would eat without going off feed. The heroic dosage employed was based on recommendations for the treatment of actinomycosis by administration of potassium iodide.

The rations of 11 of the cows (Nos. 5, 8, 12, 13, 16, 17, 18, 21, 32, 38, and 41) consisted of a grain mixture containing 17.5 per cent of protein, corn silage and alfalfa hay; while the ration of the remaining 14 cows consisted of a grain mixture containing 24 per cent of protein, corn silage and timothy hay.

Each of the cows was 3 years of age, and the greater number of them were in their second pregnancy.

NOTES ON INDIVIDUAL COWS RECEIVING IODINE

Cow No. 5 between December 4 and December 12 received 17 g of iodine; agglutination tests previous to July 17 were all negative; reaction was positive July 17, August 23, October 14, November 14, November 19, December 4, and December 13. The cow carried her calf until she was slaughtered December 13; she was due to freshen February 19. This cow received iodine eight days only.

Cow No. 8 between December 4 and January 1 received 51 g of iodine; agglutination tests were negative until April 1, when the test was doubtful; the reaction was positive April 15, negative May 1, doubtful July 17 and August 23, negative October 14 and 31, and November 14, and highly suspicious December 3. The cow calved on the date due, December 22; the calf was normal; the reaction was suspicious December 17, and slightly suspicious December 31. This cow reacted positively only once, on April 15, over seven months before iodine feeding was begun. Samples of the placenta and amniotic fluid were subjected to a test for *Bang bacillus* by injection into guinea pigs. The test showed that the organism was not present. This cow was heavily dosed with iodine for the 18 days just previous to a normal parturition.

Cow No. 10 between October 25 and November 12 received 16.47 g of iodine; agglutination tests previous to July 17 were all negative; the reaction was doubtful July 17, negative August 23, doubtful October 5, negative October 14, doubtful October 31, and negative November 14; cow aborted October 28; the calf lived a few hours only; the cow was sold for slaughter November 21. The reaction

⁴ The writers are indebted to Dr. J. F. Shigley, associate professor of veterinary science, for veterinary service rendered; and to Dr. C. J. Marshall, State veterinarian, and to his associates in the Bureau of Animal Industry, for the performance of the agglutination tests on which this report is based.

of this cow was never positive. She aborted after having had iodine for three days, about four months after the first indication, by blood test, that she might have the disease. Three days after aborting the reaction was slightly suspicious, and two weeks later was negative. Because of the variable results of the blood tests prior to iodine feeding, the last change back to negative is of doubtful significance.

Cow No. 12 between October 30 and November 25 received 26.702 g of iodine; agglutination tests were negative throughout; the cow calved normally January 1; she tolerated 1 g of iodine a day and did not go off feed.

Cow No. 11 between October 30 and November 25 received 26.702 g of iodine; agglutination tests were negative throughout; the cow calved normally November 24; she tolerated 1 g of iodine a day for 26 days.

Cow No. 15 between October 30 and November 25 received 26.702 g of iodine; agglutination test were negative throughout; the cow calved normally January 1; she tolerated 1 g of iodine daily for 26 days.

Cow No. 16 between October 25 and December 12 received 49.662 g of iodine; agglutination tests were negative prior to October 14, and doubtful October 14 and 31. The reaction was highly suspicious November 14, positive November 19, and highly suspicious on December 13. The cow was sold for slaughter December 13, while carrying a calf, and was due to freshen February 10.

Cow No. 17 between October 19 and November 9 received 41.143 g of iodine; agglutination tests were negative prior to August 23, when the test was positive; the cow aborted August 27; the reaction was positive October 14 and November 14; the cow was sold for slaughter November 17. This cow tolerated about 2 g of iodine daily for 21 days with no apparent bad effects except that less than the usual amount of hay was consumed.

Cow No. 18 between October 19 and November 9 received 42.147 g of iodine; agglutination tests prior to August 23 were all negative; the cow aborted August 1; the reaction was positive August 23, October 14 and November 14; the cow was sold for slaughter November 17. Iodine feeding at the rate of 2 g a day for 21 days brought about no change in reaction to the agglutination test. The heavy dosage of iodine caused no apparent ill effects, except that the cow consumed less than the usual quantity of hay.

Cow No. 19 between October 10 and November 25 received 26.702 g of iodine; agglutination tests were all negative; the cow freshened normally April 19. One g of iodine daily for 26 days during the fifth month of pregnancy was tolerated with no ill effects.

Cow No. 21 between October 25 and November 12 received 17.88 g iodine; agglutination tests prior to October 14 were all negative; the reaction was slightly suspicious October 14, highly suspicious October 31, and positive November 14. The cow was due to calve November 24, but aborted October 31, when iodine feeding had been in progress 6 days, and was sold for slaughter November 17. The reaction changed from slightly suspicious to positive while the cow was receiving iodine at the rate of 1 g daily.

Cow No. 23 between October 30 and November 25 received 26.702 g of iodine; agglutination tests were all negative prior to November 19 when the reaction was highly suspicious. The cow aborted November 28, being due to calve February 10, and was sold for slaughter November 29. This animal was apparently infected and was incubating the disease before the iodine feeding was started. After being on the iodine (1 g daily) for 19 days she reacted in a manner designated highly suspicious, and aborted 9 days later, 3 days after the close of a 26-day iodine feeding period.

Cow No. 25 between October 30 and November 25 received 26.702 g of iodine; agglutination tests were all negative. The cow when examined on November 27 was found to be pregnant, and freshened normally on April 11. This cow (negative to abortion test) was fed 1 g of iodine daily for a 26-day period with no ill effects, in the fourth and fifth months of pregnancy.

Cow No. 26 between October 30 and November 25 received 26.702 g of iodine; agglutination tests were all negative; the cow aborted December 10; the calf was 6 weeks premature. This cow was sold for slaughter December 13. A blood sample taken on date of slaughter was found negative. The cow aborted 18 days after the close of a 26-day iodine feeding period. A positive blood reaction had not developed 3 days thereafter.

Cow No. 28 between October 19 and November 9 received 33.957 g of iodine; agglutination tests prior to July 17 were all negative, but were positive July 17 and August 23. The cow aborted September 12; the calf was born dead; the cow was due to calve in January. The reaction was positive October 14 and November 14; the cow was sold for slaughter November 17. She had aborted

about one month previous to a 21-day period of iodine feeding. Iodine feeding ($1\frac{1}{2}$ g daily) produced no change in reaction.

Cow No. 31 between October 19 and November 9 received 13.621 g of iodine; agglutination test August 23 was highly suspicious; and positive October 5, October 14 and November 14. The cow aborted October 4, the calf being dead, and was sold for slaughter November 15. This cow aborted two weeks previous to beginning of 21-day iodine feeding period and would tolerate only a small quantity of iodine.

Cow No. 32 between October 25 and January 1 received 48.662 g of iodine; agglutination tests were all negative until October 5 when the test was designated slightly suspicious; on October 14 it was highly suspicious, positive October 31, highly suspicious November 14, and positive November 19 and December 3. The cow aborted December 11, and had living calf weighing 45 pounds, one month premature, which lived until December 23. The reaction of the cow was positive December 17 and December 31. This cow was sold for slaughter January 7, 1930. The reaction changed from positive to highly suspicious about three weeks after iodine feeding was started, then changed to positive, and the cow aborted when the iodine feeding had been in progress 47 days. Iodine feeding was then continued 20 days longer with no change in the blood reaction.

Cow No. 34 between October 30 and November 25 received 26.702 g of iodine; agglutination tests were negative until November 19, when the cow reacted in a manner designated slightly suspicious; the reaction was positive December 3 and 13; cow aborted December 7, but the calf lived only 12 hours. The cow was due to calve December 27, and was sold for slaughter December 13. The blood test when iodine feeding was started was negative but changed to slightly suspicious when the cow had received iodine 20 days, and two weeks later the reaction was positive. Four days later the cow aborted, 12 days after the close of 26-day iodine feeding period.

Cow No. 35 between October 19 and November 25 received 40.303 g of iodine; agglutination tests were negative prior to May 1, on which date test was positive; they were also positive July 17, August 23, October 14, and November 14 and 19; the cow would not conceive and was sold for slaughter November 29. A 36-day iodine feeding period, in which a total of 40 g of iodine was fed, produced no change in the positive blood reaction.

Cow No. 37 between October 19 and November 9 received 42.639 g of iodine; agglutination tests were negative prior to July 17, on which date the reaction became positive. The cow was not pregnant. Tests were positive August 23, October 14, and November 14. The cow was sold for slaughter November 15. A daily dose of over 2 g of iodine for 20 days made no change in the blood reaction. The cow ate less than the normal quantity of hay.

Cow No. 38 between December 4 and December 12 received 18 g of iodine; agglutination tests were negative prior to April 15, on which date the test was positive; it was positive May 1, August 23, October 23, November 14 and 19, and December 13. The cow was due to calve January 8, but was sold for slaughter December 13. With eight days of iodine feeding, at the rate of $2\frac{1}{4}$ g a day, during the eighth month of pregnancy, there was no change in blood test.

Cow No. 39 between October 25 and November 25 received 31.662 g of iodine; agglutination tests were negative until October 5, on which date the test was slightly suspicious; it was negative October 14 and 31, and November 14, and slightly suspicious November 19. The cow was sold for slaughter November 29. This cow had an abscessed ovary and would not conceive. Her blood test was never more than slightly suspicious. Thirty-one days of iodine feeding (1 g daily) produced no change in reaction.

Cow No. 40 between October 29 and December 13 received 70.817 g of iodine; agglutination tests were negative until August 23, on which date the test was positive; it was also positive October 14 and November 14, highly suspicious November 19, and positive December 3 and 13. The cow was due to calve January 18, but was sold December 13. A 45-day iodine feeding period, in which a total of over 70 g of iodine were fed, produced no change of blood reaction.

Cow No. 41 between October 30 and November 25 received 26.702 g of iodine; agglutination tests were negative prior to November 14, on which date test was slightly suspicious, and again negative November 19. The cow was sold November 29 because of failure to conceive. Reaction was negative at the time iodine feeding was started, but slightly suspicious after the cow had received iodine for two weeks. Two weeks later the reaction changed back to negative.

Cow No. 44 between October 19 and January 1 received 82.755 g. of iodine; agglutination tests were negative until August 23, when the test was highly suspicious; tests were also highly suspicious October 5, 14, and 31, and November

14, positive November 19 and December 3, 17, and 31. The cow calved at full term December 19, and the calf was apparently healthy. The blood test on this cow was highly suspicious when iodine feeding was started, but changed to positive after the cow had been given iodine for one month, and continued positive until the cow was sold. Iodine was given, in doses of more than 1 g a day for 73 days including the last 60 days previous to a normal, full-time parturition.

DISCUSSION OF RESULTS

Iodine was first administered at the rate of 5 g of the element a day, in the form of iodized linseed meal. This quantity threw the cows off feed on the second day, but it was found that most individuals would tolerate from 2 to 3 g of iodine a day, at least for a limited period. Certain cows tolerated 2 g of iodine daily, as long as it was fed to them, while others would not tolerate more than 1 g a day. Some of the cows resented the presence of the iodized linseed meal in their ration from the beginning, while others paid little attention to it and appeared to eat their grain with the usual relish. After receiving a heavy dosage of iodine for several days, there was in all cases a decrease of appetite for hay. Possibly the iodine interfered with essential fermentations in the rumen.

After several days of heavy iodine feeding the cows became sluggish; the hair grew rough, and in certain individuals there was copious nasal discharge, and brownish discoloration of the skin about the eyes, nose, and tail.

The cream produced by the cows that received iodine was rejected by the inspector at the college creamery, because of its objectionable odor; and analysis of the cream, and also of the mixed milk of cows Nos. 5, 16, and 38, revealed the presence of iodine in very large quantities.

At the time this investigation was started it was the plan to continue the iodine feeding for several months, but conditions which developed thereafter rendered it desirable to dispose of all animals infected with abortion disease without delay. Two cows, Nos. 5 and 38, were fed iodine for only eight days. The average length of time during which the 23 others received iodine was 30½ days. Of these cows 6 received iodine for more than 30 days and the remaining 17 received iodine for an average of 23.6 days. The average total quantity of iodine fed these 17 cows was 31.7 g. Three cows, Nos. 16, 32, and 44, were fed for an average of 63 days, the average total of iodine given being 63.7 g.

THE EFFECTS OF IODINE ON THE GROWTH AND PHYSICAL CONDITION OF DAIRY CALVES

The object of this investigation was to learn whether iodine, especially in the form of iodized linseed oil meal, is of value in the rearing of dairy calves.

The calves which served as subjects were grade Holstein females which were purchased at ages of 1 to 5 days in groups of 4 to 12—as they were available—from dairy farmers in Bradford County. Great care was taken to select vigorous animals of uniform size, and they were reared in accordance with the usual practice on good dairy farms.

In the conduct of the experiment the identity of the groups of the calves as purchased was maintained, for convenience, each group being

divided into pairs, as evenly balanced as possible, one animal of each pair receiving iodine and the other serving as a control.

The calves received whole milk until they were 2 to 3 weeks of age. The ration was then gradually changed to skim milk, grain, and hay. The two calves of each pair were fed exactly the same quantities of feeds (with the exception of hay) throughout the experiment, the quantities allowed to each pair being determined by the calf with the smaller appetite.

The first four or five weeks' feeding constituted a preliminary period. During this time the calves were established on the experimental ration, and the feeding was carried out in the same systematic manner as during the later experimental feeding.

The grain mixture was made up of 75 pounds of oats, 75 pounds of wheat bran, 40 pounds of yellow hominy feed, 40 pounds of yellow corn meal, 50 pounds of linseed meal, and 1½ pounds of salt.

Four kinds of hay were fed, at different times during the experiment, viz, early cut timothy, high-grade New Jersey alfalfa, mixed hay of good quality, and high-grade red clover. The respective proportions of the total hay fed were 4, 15, 22, and 59 per cent. The timothy was fed during the preliminary period because of its usefulness in the prevention of digestive disturbances in young calves.

Each calf was given as much hay as it would eat. With each pair of calves, without exception, the one receiving iodine ate less hay than did the control.

The calves were weighed once each week, and a measurement of the height at the withers was taken once a month. The average daily intake of feeds and the total cod-liver oil and iodine fed are shown in Table 2.

TABLE 2.—Average daily feeds and total cod-liver oil and iodine eaten by calves

Group No., number of days on experiment, and sub-group	Average daily skim milk	Average daily grain	Average daily hay	Total cod-liver oil per calf	Total iodine per calf
	Pounds	Pounds	Pounds	Cc	Grams
Group 1, 139 days:					
3 calves receiving iodine	16.73	2.04	1.55	283.3	2.570
3 calves receiving no iodine	16.73	2.04	1.83	283.3	None.
Group 2, 131 days:					
6 calves receiving iodine	16.49	2.13	1.79	265.0	2.409
6 calves receiving no iodine	16.49	2.13	1.85	265.0	None.
Group 3, 117 days:					
4 calves receiving iodine	16.20	2.34	1.89	255.0	2.759
4 calves receiving no iodine	16.20	2.34	2.16	255.0	None.
Group 4, 110 days:					
3 calves receiving iodine	15.07	1.99	1.14	190.0	1.788
3 calves receiving no iodine	15.07	1.99	1.44	190.0	None.
Group 5, 103 days:					
2 calves receiving iodine	15.46	1.68	1.68	190.0	1.615
2 calves receiving no iodine	15.46	1.68	1.51	190.0	None.
Group 6, 75 days:					
3 calves receiving iodine	14.48	2.19	1.35	260.0	.727
3 calves receiving no iodine	14.48	2.19	1.31	260.0	None.

The analysis of the ration indicated a normal iodine intake considerably greater than the 1 mg weekly which Marine⁵ reported as adequate for thyroid maintenance in dogs. This estimate of the iodine content of the ration disregards the analysis of one grain mixture, a sample of which showed one hundred times the amount of iodine present in other foods of similar type. Unfortunately the

⁵ MARINE, D., and KIMBALL, O. P. Op. cit.

analysis was made after the feeding with the questioned material had been completed, and it was impossible to determine whether the extremely high iodine content was continuously present or whether the sample was unaccountably contaminated at the time of taking.

The quantity of iodine fed was adjusted once a week, the standard allowance being 30 mg per hundred pounds live weight. The iodine was fed once a day in the skim milk. After they had received iodine for about four weeks, the calves displayed marked evidence of having had too much of it. Their coats became very rough, and they did not eat with the usual appetite. The feces were thin and of an abnormal reddish-brown color. There was considerable variation in the tolerance of the individuals for iodine, and the appetites of some were so much affected that they ate only a small proportion of the normal quantity of hay. The administration of iodine, therefore, was discontinued for intervals of from 17 to 44 days, in the several groups, after which it was resumed at a lower level of intake, namely, 10 mg per hundred pounds live weight a day.

Table 3 gives the intervals of time during which iodine was administered or was withheld.

TABLE 3.—Schedule of iodine feeding

Group	Interval during which iodine was fed from beginning of experiment	Interval during which iodine was not fed	Final interval during which iodine was fed at diminished rate to end of experiment	Group	Interval during which iodine was fed from beginning of experiment	Interval during which iodine was not fed	Final interval during which iodine was fed at diminished rate to end of experiment
	Days	Days	Days		Days	Days	Days
No. 1.....	43	44	50	No. 4.....	34	34	22
No. 2.....	43	44	43	No. 5.....	28	33	30
No. 3.....	48	17	65	No. 6.....	6	43	27

The iodine was omitted from the feed of four calves before the end of the investigation, as indicated by the next to the last column in Table 4.

The very poor physical condition of some of the calves that had received iodine suggested the desirability of special measures for restoring them to health. Cod-liver oil was therefore used, both calves of each pair receiving the same quantity—usually 10 c c a day. The cod-liver oil was continued, with each pair of calves, as long as it seemed to be needed by the calf that had received iodine. The average length of time during which the calves were given cod-liver oil was 31 days, although 2 received it for 72 days, 4 received it for 58 days, and 6 received it for 41 days.

During the final period of administering iodine the drug was given to 11 of the calves in the form of potassium iodide, instead of iodized linseed meal, in order to reveal possible differences in the response of the animals to iodine in the two combinations.

On the lower plane of iodine intake there was no definite evidence of iodine excess, and during the brief period of comparison of iodized linseed meal and potassium iodide no differences could be detected of their effect on the calves.

TABLE 4.—Gains of calves in weight, and in height at withers

Group and number of days on experiment	Controls, which received no iodine				Calves which received iodine					
	Calf No.	Initial weight	Gain in weight	Gain in height at withers	Calf No.	Initial weight	Gain in weight	Gain in height at withers	Period fed iodine	Total iodine fed
		Lbs.	Lbs.	Centi-meters		Lbs.	Lbs.	Centi-meters	Days	Grams
Group 1, 139 days.....	77	94	197	21.5	76	92	205	24.0	93	2.458
	78	100	219	23.0	80	96	190	24.5	93	2.665
	79	105	218	22.5	81	108	199	24.0	93	2.587
Average.....		99.6	211.3	22.3		98.6	198.0	24.1		
Group 2, 131 days.....	83	115	216	24.5	89	120	208	23.5	86	2.774
	85	100	193	17.0	95	93	156	21.0	83	1.982
	87	115	191	25.0	84	113	211	28.0	86	2.676
	86	90	189	24.0	91	89	217	24.0	86	2.323
	94	106	215	25.0	93	110	216	23.0	86	2.665
	88	88	177	20.0	82	87	164	21.0	86	2.037
Average.....		102.3	196.8	22.5		102.0	195.3	23.4		
Group 3, 117 days.....	101	101	183	17.0	100	104	176	19.0	99	3.052
	77	100	193	18.0	99	93	191	18.0	99	2.416
	96	113	178	18.5	102	105	165	19.0	99	2.551
	98	116	203	20.5	103	118	219	22.0	99	3.016
Average.....		107.5	189.2	18.5		105.0	187.7	19.6		
Group 4, 110 days.....	112	93	152	20.0	106	90	140	15.0	53	1.090
	108	110	132	18.0	111	102	141	19.0	76	2.111
	110	128	147	18.0	107	110	151	17.5	76	2.164
Average.....		110.3	143.6	18.6		100.6	144.0	18.1		
Group 5, 103 days.....	120	100	128	11.0	118	98	131	14.0	68	1.531
	117	110	161	18.0	114	112	160	17.0	60	1.700
Average.....		105.0	144.5	14.5		105.0	145.5	15.5		
Group 6, 75 days.....	122	111	126	15.0	121	119	130	15.0	32	.758
	127	100	117	14.0	125	109	116	15.0	32	.671
	126	105	107	12.0	123	105	111	18.0	32	.752
Average.....		105.3	116.6	13.6		111.0	119	16.0		

The gains in weight of the calves, as recorded in Table 4, do not indicate a significant difference in growth between those that received iodine and those that did not. Among the 21 pairs of calves, in 10 cases the calf receiving iodine made the greatest gain. The total increase in weight of the 21 calves receiving iodine was 45 pounds less than the total gain of the 21 calves that received no iodine. The mathematical interpretation of the differences in gains in weight, by Student's method, revealed odds of 2.8 to 1 indicating that the calves which did not receive iodine had made the greater average gain in weight. These odds are not statistically significant.

A similar interpretation of the gains in height at withers gives results favorable to iodine in five of the six groups. The separate consideration of the 13 pairs of Groups 1, 2, and 3, which received larger total quantities of iodine than did the remaining groups, gave odds of 65 to 1 in favor of the indication that the calves receiving iodine made the greater gains in height; while the consideration of the entire 21 pairs of calves gave odds of 77 to 1 in favor of the same possibility. These odds are statistically significant.

The cause of the apparent stimulation of skeletal growth, as a result of the administration of iodine, was not revealed. This response of the calves receiving iodine is especially puzzling because they ate appreciably less hay than did those that received no iodine—the hay containing a very considerable part of the calcium of the ration.

While there is ground for question as to the iodine intake of these calves, on account of the excessively high apparent iodine content of the grain mixture fed, it appears that the tolerance of calves for iodine is less than that of sheep, swine, and chickens.

It also seems that the iodine of iodized linseed meal, which was fed during the greater part of the experiment, produced a more marked reaction on the calves than does the iodine of iodides.

At the time of this writing, the calves now being about 1 year of age, the difference in height of the calves which received iodine, as compared with those that received none, is no longer apparent, and that is no apparent difference in health of the calves as a result of iodine feeding.

THE VALUE OF SUPPLEMENTAL IODINE IN THE GROWTH OF SWINE

Forty purebred pigs of the Berkshire, Chester White, Duroc-Jersey, and Poland China breeds were divided into 10 lots of four each, as nearly as possible of the same size, sex, age, and breed representation.

Five lots were given iodine in addition to the basal ration (Table 5), while the other five were used as controls or checks.

TABLE 5.—*Live weights, gains in weight, and feed consumption of 10 lots of 4 pigs each, alternate lots receiving supplemental iodine with the ration for 126 days*

Lot and treatment	Average initial weight	Average final weight	Average daily gain	Feed per 100-pound gain
	Pounds	Pounds	Pounds	Pounds
No. 1, iodine.....	50.6	223.0	* 1.4	495.7
No. 6, check.....	48.7	221.0	1.37	426.7
No. 2, iodine.....	54.7	234.7	1.43	417.9
No. 7, check.....	55.2	221.7	1.32	447.7
No. 3, iodine.....	42.2	194.7	1.21	412.0
No. 8, check.....	40.5	165.7	.99	498.0
No. 4, iodine.....	41.2	188.0	1.16	335.5
No. 9, check.....	41.0	179.0	1.09	347.3
No. 5, iodine.....	51.5	188.7	1.08	345.5
No. 10, check.....	52.8	209.0	1.23	320.0

* Adjusted on account of the removal of 1 pig in the course of the experiment.

The composition of the rations is shown in Table 6. The rations were hand fed, the feeds being dry, to the limit of the pigs' appetites. The iodine was fed in the form of iodized linseed meal thoroughly mixed into the ration, once each day, at a rate of 50 mg of the element per hundred pounds live weight.

TABLE 6.—*Composition of basal ration of pigs*

Constituent	Proportions in which feed was mixed		Constituent	Proportions in which feed was mixed	
	First 112 days	Last 14 days		First 112 days	Last 14 days
Ground yellow corn.....	160	180	Tankage.....	20	20
Ground oats.....	40	40	Linseed meal.....	10	10
Wheat middlings.....	40	40	Salt.....	1	1

The age of the pigs at the beginning of the trial ranged from 10 to 12 weeks, and their average initial weight, in the several lots (Table 5), ranged from 40.5 to 55.2 pounds.

The test was continued until the average live weight per lot, in each group of five lots, reached approximately 200 pounds, which required 126 days with each group.

The initial and final weights, recorded in Table 5, are each the average of three weights taken on consecutive days. In addition, intermediate, individual weights were taken at 2-week intervals.

This experiment was conducted between December 3, 1929, and April 8, 1930. The pigs were confined in dirt lots in which there was no vegetation and were sheltered by small movable houses. It was possible, therefore, for the pigs to supplement the inorganic nutrients of the rations to a limited extent by eating earth.

No effects of the iodine feeding were observable in the performance of the pigs.

The essential data of the experiment are given in Table 5.

THE VALUE OF SUPPLEMENTAL IODINE IN THE FATTENING OF LAMBS

Two feeding experiments, one with 30 lambs fed individually by the paired feeding method, and a second with 171 lambs fed in six approximately equal groups, were conducted to learn whether iodine, in the form of iodized linseed meal, is of value in the fattening of lambs.

LAMBS FED BY THE PAIRED-FEEDING METHOD

The lambs were raised in northern Pennsylvania and were divided into 15 pairs, of the same sex, and as nearly as possible of the same weight and of the same condition as to fatness.

Each lamb was fed in the same individual crate throughout the entire experiment, which was started December 31, 1929, and ended March 18, 1930, a period of 77 days. Both members of each pair were given the same quantity of feed; and if one of a pair refused a part of its feed, the ration of its mate was diminished in like proportion.

The ration was one which is commonly used for fattening lambs, and consisted of alfalfa hay and a grain mixture, the latter composed of 9 parts of coarsely ground corn, 1 part of linseed meal, and 0.5 per cent of salt. The analysis of this basic ration showed iodine to be present in amounts usually considered adequate for the maintenance of thyroid function. In addition one member of each pair was given 33 mg of iodine per hundred pounds of live weight, in the form of iodized linseed meal, the iodine being fed once each day thoroughly

mixed with the grain ration. An earlier effort to feed the same total quantity of iodine in two doses a week was unsuccessful, since it sickened the lambs. The daily feeding, however, produced no unfavorable effects.

The initial and final weights are averages of three weighings each, taken on as many consecutive days, and in addition the lambs were weighed each week. The initial weights of the lambs ranged from 56 to 72 pounds, and the final weights from 72 to 105 pounds.

The lambs were fed in a closed shed on a ground floor bedded with straw, some of which was eaten. During winter days, when the ground was covered with snow, the lambs were permitted to run outside for a few hours each day. The lambs were not treated for parasites. Several were slaughtered, and all of these, especially lamb No. 2, were found to be infested with the nodule parasite.

The numerical data of the experiment are presented in Table 7.

TABLE 7.—Live weights, gains in weight, and feed consumption of 15 pairs of lambs, one individual of each pair receiving supplemental iodine with the ration for 77 days

Pair and treatment	Initial weight	Final weight	Average daily gain in weight	Average daily ration	Feed consumed, per pound of gain in weight
	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>
No. 1:					
Iodine.....	63	96	0.43	2.74	6.40
Check.....	65	99	.44	2.76	6.25
No. 2:					
Iodine.....	63	72	.12	2.16	18.51
Check.....	69	90	.27	2.16	7.92
No. 3:					
Iodine.....	72	104	.41	2.66	6.41
Check.....	72	105	.43	2.67	6.22
No. 4:					
Iodine.....	60	73	.16	1.75	10.37
Check.....	60	69	.12	1.78	15.23
No. 5:					
Iodine.....	60	89	.38	2.51	6.68
Check.....	56	85	.38	2.53	6.72
No. 6:					
Iodine.....	66	96	.39	2.54	6.53
Check.....	64	90	.34	2.54	7.55
No. 7:					
Iodine.....	61	83	.28	2.49	8.73
Check.....	66	90	.31	2.49	8.00
No. 8:					
Iodine.....	62	82	.26	2.46	9.49
Check.....	59	83	.31	2.48	7.97
No. 9:					
Iodine.....	69	89	.26	2.46	9.47
Check.....	68	90	.28	2.47	8.65
No. 10:					
Iodine.....	64	87	.30	2.10	7.06
Check.....	61	74	.16	2.10	12.42
No. 11:					
Iodine.....	68	90	.28	2.28	7.97
Check.....	61	81	.26	2.28	8.77
No. 12:					
Iodine.....	59	85	.34	2.33	6.91
Check.....	67	92	.32	2.33	7.11
No. 13:					
Iodine.....	57	80	.30	2.45	8.23
Check.....	56	78	.28	2.45	8.69
No. 14:					
Iodine.....	59	77	.23	2.23	9.55
Check.....	56	77	.27	2.25	8.28
No. 15:					
Iodine.....	69	99	.39	2.77	7.13
Check.....	63	96	.43	2.77	6.48

LAMBS FED IN GROUPS^a

The group-feeding experiment was conducted between November 2, 1929, and February 14, 1930. The lambs used in this experiment included native fine wools grown in southwestern Pennsylvania, native muttons from northern Pennsylvania, and western lambs grown in the State of Washington. The lambs from each source were divided into 2 groups of 28 or 29 each, and 1 group from each of the 3 sources was fed, in addition to the regular ration, 33 mg of iodine per 100 pounds live weight daily for 104 days. The iodine was fed twice daily in the form of iodized linseed meal thoroughly mixed with the grain ration.

The average initial weights of the lambs in the lots ranged from 52.3 to 55.9 pounds. The average final weights ranged from 71.6 to 91.4. (Table 8.) The ration consisted of mixed hay, hand fed twice daily, and a grain mixture of 9 parts corn and 1 part linseed meal likewise fed twice each day.

TABLE 8.—*Live weights, gains in weight, and feed consumption of six groups of lambs, three of which received supplemental iodine with their ration for 104 days*

Type of lambs, group, and treatment	Lambs at start	Lambs at end ^a	Initial weight (average)	Final weight (average)	Daily gain (average)	Feed intake per 100 pounds of gain			
						Shelled corn	Oil cake	Hay	Total
Native fine wool:	Number	Number	Pounds	Pounds	Pounds	Pounds	Pounds	Pounds	Pounds
No. 1, iodine.....	29	27	55.9	73.0	0.16	530.4	59.7	738.3	1,328.4
No. 2, check.....	28	27	55.8	75.9	.19	461.9	50.7	631.3	1,143.9
Native mutton:									
No. 3, iodine.....	29	27	52.3	71.6	.18	500.8	52.3	648.7	1,201.8
No. 4, check.....	28	27	53.1	74.4	.21	450.2	49.9	565.3	1,065.4
Western:									
No. 5, iodine.....	29	27	54.1	86.6	b, 26	403.5	44.8	491.9	940.2
No. 6, check.....	28	27	55.6	91.4	c, 28	385.0	41.6	467.8	894.3

^a 1 or 2 lambs in each group were slaughtered in the course of the experiment for carcass studies.

^b Adjusted on account of the removal of 2 lambs in the course of the experiment.

^c Adjusted on account of the removal of 1 lamb in the course of the experiment.

The lambs receiving iodine cleaned up their feed less promptly than did those receiving no iodine.

THE VALUE OF SUPPLEMENTAL IODINE FOR CHICKENS

In the experiment to determine the effects of the administration of supplemental iodine to chickens during growth and early egg production, four groups of single-comb white leghorn female chicks, containing 160, 170, 184, and 187 individuals, respectively, were fed the experimental rations from the time of hatching to the age of 32 weeks.

Groups 1 and 2 received the same varied mash ration, the animal-protein components of which were fish meal, meat scrap, and dried milk. Group 2 received in addition iodized linseed meal in a quantity sufficient to provide 50 mgs of iodine per 100 pounds of chicken a day.

The mash ration for Groups 1 and 2 consisted of 40 pounds of yellow corn meal, 15 pounds of wheat bran, 15 pounds of flour wheat middlings, 10 pounds of alfalfa-leaf meal, 10 pounds of dried milk, 5

^d This report is based on an experiment conducted under the direction of Associate Prof. W. L. Henning, primarily as a comparison of types of lambs as feeders.

pounds of fish meal, 5 pounds of meat scrap, 2 pounds of steamed bone meal, 1 pound of salt, and 1 pound of cod-liver oil.

Groups 3 and 4 received a mash ration similar to that fed to Groups 1 and 2, except that the fish meal was replaced by meat scrap, Group 4 receiving this mash ration by itself, and Group 3 the same plus iodine, given at the same rate as to Group 2.

For the first 8-week period the iodized linseed meal was fed in accordance with predetermined standard live weights;⁷ and thereafter in accordance with the experimental weighings which were taken individually every four weeks.

In addition to the mash rations all groups received a half-and-half cracked corn and wheat scratch mixture beginning with the fifth week and continuing to the end of the experiment.

The four groups of chicks used in the experiment were hatched at weekly intervals as follows: Group 1, March 20; Group 2, March 27; Group 3, April 3; Group 4, April 10, 1930. All males were removed at eight weeks of age, the data reported here being for females only. The chicks were placed in brooding quarters when 24 hours old and were fed the proper mash ration. The same mash mixture was fed, ad libitum, in hoppers, during the entire course of the experiment; and the scratch grain mixture, given after the fourth week, was also fed in hoppers.

During the first 16 weeks, the birds were housed in a long type of brooder house, each pen of which is 12 by 20 feet. Each group had access to a sun porch 8 by 10 feet in size. At the end of the 16-week period they were moved into laying quarters and remained there until the conclusion of the experiment. At no time in the course of the experiment were the birds allowed out on range.

Cannibalism was not manifest in an unusual degree in any of the groups during the experiment, the infrequent occurrence of this habit being approximately evenly distributed among the four groups.

No difference was noticeable in the behavior or the condition of the four groups of birds at any time during the experiment.

The average growth, the number of birds culled at the age of 16 weeks, the mortality, and the average number of eggs laid, for each group are given in Table 9.

TABLE 9.—*The effect of iodine on the growth, mortality, and early egg production of chickens*

Group and treatment	Age	Birds in groups	Average body weight	Mortality		Average number of eggs laid to age of 32 weeks
				Per period	Total	
	Weeks	Number	Grams	Per cent	Per cent	
No. 1, no iodine.....	0	160	38			31
	4	160	232	0	0	
	8	160	508	0	0	
	12	159	817	0.6	0.6	
	16	155	1,030	2.5	3.1	
	20	131	1,274	0	3.1	
	24	122	1,462	5.6	8.7	
	28	114	1,531	5.0	13.7	
	32	109	1,589	3.1	16.8	

* 24 culled at age of 16 weeks.

⁷ CHARLES, T. B., and KNANDEL, H. C. REARING CHICKS IN CONFINEMENT. Penn. Agr. Expt. Sta. Bul. 218, p. 11, Table III. 1928.

TABLE 9.—*The effect of iodine on the growth, mortality, and early egg production of chickens—Continued*

Group and treatment	Age	Birds in group	Average body weight	Mortality		Average number of eggs laid to age of 32 weeks
				Per period	Total	
	Weeks	Number	Grams	Per cent	Per cent	
No. 2, iodine.....	0	170	40			
	4	170	235	0	0	
	8	169	523	0.6	0.6	
	12	167	796	1.2	1.8	
	16	142	1,124	1.2	3.0	35
	20	138	1,368	2.4	5.4	
	24	130	1,516	4.7	10.1	
	28	125	1,656	2.9	13.0	
	32	121	1,674	2.4	15.4	
No. 3, iodine.....	0	184	39			
	4	182	211	1.1	1.1	
	8	181	450	.5	1.6	
	12	174	711	3.8	5.4	
	16	146	983	1.6	7.0	24
	20	138	1,221	4.3	11.3	
	24	135	1,408	1.6	12.9	
	28	134	1,585	.5	13.4	
	32	130	1,764	2.2	15.6	
No. 4, no iodine.....	0	187	38			
	4	187	209	0	0	
	8	182	451	2.7	2.7	
	12	179	718	1.6	4.3	
	16	158	970	1.6	5.9	24
	20	148	1,220	5.3	11.2	
	24	133	1,451	8.0	19.2	
	28	132	1,531	.5	19.7	
	32	123	1,729	4.8	24.5	

^b 23 culled at age of 16 weeks; also 2 died.

^c 25 culled at age of 16 weeks; also 3 died.

^d 18 culled at age of 16 weeks; also 3 died.

Attention is called to the fact that Group 2, which received iodine, excelled Group 1 in body weight, after the twelfth week; but doubt is thrown upon the significance of this observation by the fact that Group 3, which received iodine, was excelled by Group 4 in body weight.

SUMMARY

Twenty-five grade Holstein milking cows which were infected with, or had been exposed to, contagious abortion were subjected to liberal dosage with iodine in the form of iodized linseed meal. The length of time of feeding the different individuals varied from 8 to 73 days, the average being 28.7 days. The average total amount of elemental iodine fed was 34.32 g per cow. The average daily dose per cow was 1.2 g. Individual cows varied in their ability to tolerate iodine from 0.65 g to 2.13 g a day. The maximum total amount fed any individual was 82.8 g in a period of 73 days.

Agglutination blood tests for abortion disease were made at 2-week intervals during the iodine feeding, monthly tests having been conducted previous to that time.

The administration of iodine did not affect the condition of disease, as indicated by results of the agglutination blood test. The disease developed in the normal manner, in five cows, the tests progressing from suspicious to positive during the time iodine feeding was in progress. There were no clear-cut cases of change in reaction in the reverse direction. In the case of five cows of the six that aborted during the iodine treatment, the development of the disease was clearly indicated

by the blood test. Six animals furnished rather conclusive evidence that intensive iodine feeding during the last six months of pregnancy was not harmful. No cows were fed iodine during the first three months of pregnancy.

The milk and cream produced during heavy iodine feeding had an objectionable odor and contained very large quantities of iodine.

Forty-two calves were fed, by the paired system of feeding, in an effort to learn whether iodine, especially in the form of iodized linseed meal, is of value in the rearing of dairy calves.

The duration of the experimental feeding of the different pairs of calves ranged from 75 to 139 days. Twenty-six of the calves were fed for 117 days or longer.

The ration was made up of skim milk, grain, and hay, as in good average practice. Cod-liver oil was included in the ration of all calves during a part of the time.

Iodine was fed to one calf in each pair in quantities ranging from 10 mg to 23.5 mg a day. Thirteen individuals received 18.5 mg or more a day, as an average for the experiment.

Thirty milligrams of iodine per hundred pounds of live weight, in the form of iodized linseed meal, proved to be a heavier dosage than the calves could tolerate, as revealed by loss of appetite (particularly for hay), roughness of the hair, digestive disturbances, and an emaciated condition, after receiving iodine at this rate for from three to five weeks.

With few exceptions, the calves could tolerate 10 mg of iodine, in the form of iodized linseed meal, per hundred pounds live weight, with no harmful effect.

The calves that received iodine ate less hay and made appreciably less gain in weight, but greater gain in height, on an average, than did those receiving no iodine.

The addition of cod-liver oil to the ration of the calves, when they were in an emaciated condition as a result of excessive iodine intake, proved very helpful in bringing the calves back to a normal condition.

Among 10 experimental lots of growing swine, fed as matched pairs of lots, three lots receiving iodine made greater gains in weight than did their check lots, while two check lots made greater gains than did the paired lots receiving iodine. The differences in three cases were too small to be of possible significance.

The five groups that received iodine required an average of 401.3 pounds of feed to produce 100 pounds of gain in weight, while the five groups used as checks required 407.9 pounds of feed to produce 100 pounds of gain.

It is therefore evident that no certain beneficial results, either as to amount or cost of gain in weight, were derived from feeding iodine, in the form of iodized linseed meal, to growing swine.

Fifteen carefully selected pairs of native Pennsylvania lambs were fed for 77 days in a study of the value of supplemental iodine in the fattening of lambs.

One lamb of each pair received in addition to its ration 33 mg of iodine a day in the form of iodized linseed meal, while the other lamb constituted a control or check.

Among the 15 pairs of lambs, in 7 pairs the lamb receiving iodine gained in weight the more rapidly, while in the remaining 8 pairs the check lamb gained the more rapidly.

Of 165 comparisons between weekly gains in live weight of the pairs, 63 favored the lamb which received iodine; 69 favored the check lamb, and 33 comparisons were of neutral significance.

The average difference between the average daily gains of pair mates was 0.01 pound in favor of the check lamb.

These results show clearly that iodine as fed in this experiment produced no appreciable effect in the fattening of lambs.

The average daily gains in live weight of the lambs in the groups which received iodine were slightly less than those in the check or control groups, the difference ranging from 0.02 to 0.03 pound.

The feed required for 100 pounds of gain in live weight was greater for the lots that received iodine. The iodine fed groups of the native fine-wool lambs, the native mutton lambs, and the western lambs required 184.5, 136.4, and 46.9 pounds more feed, respectively, for each 100 pounds of gain than was required by the check lots.

There was, therefore, a slight detrimental effect from the feeding of iodine to these lambs, and this effect was greater with lambs from the State of Pennsylvania than with those from the State of Washington.

Four groups of single comb white leghorn female chicks, containing from 160 to 187 individuals each, were fed from hatching time to the age of 32 weeks to determine the value of supplemental iodine in relation to growth and early egg production.

Two of the groups received a normal mixed ration containing fish meal, while the others received a similar ration but with meat scrap replacing the fish meal.

The iodine was administered in the form of iodized linseed meal at a rate supplying 0.05 of iodine per 100 pounds of chicken a day.

No certain effect of the iodine on the growth, mortality, or egg laying of the birds was observed.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 45

WASHINGTON, D. C., AUGUST 1, 1932

No. 3

THE RÔLE OF NITROGEN IN THE PRODUCTION OF SPOTS IN WHEAT FIELDS¹

By P. L. GAINEY, *Soil Bacteriologist*, and M. C. SEWELL, formerly *Associate in Soils, Kansas Agricultural Experiment Station*

INTRODUCTION

During the past 20 years the Kansas Agricultural Experiment Station has devoted a great deal of attention to problems relating to soil nitrogen and has endeavored to follow any clue which it was felt might throw light on the relation of nitrogen to wheat production, particularly in regions of relatively little rainfall.

In the small-grain fields of Kansas there are frequently observed well-defined spots on which the grain is darker green and more vigorously growing than that on the surrounding areas. So abundant are these spots in the eastern half of Kansas that some fields present a polka-dot appearance, as shown in Figure 1. These spots are ordinarily 2 to 3 feet in diameter and circular or oblong in shape. In a typically spotted field when the grain is in the booting stage such spots are easily visible a half mile away. They are more frequently observed in fields that have been grazed, and are commonly attributed to deposits of feces or urine. They seem to occur upon heavy, potentially fertile soil as frequently as upon light, nonfertile soil, particularly if the season has been wet and cold. The general appearance of the spots, together with their supposed origin, indicated that they might in some way be connected with the nitrogen metabolism of the plant. The studies of Lipman,² who first called attention to these spots, confirmed this belief.

Some preliminary studies were conducted by the writers as early as 1916, and in the spring of 1929, 1930, and 1931 the studies were extended as far as it was felt the problem and facilities would justify. The object of these studies was to determine (1) the rôle, if any, that available nitrogen plays in the production of the spots; (2) in case available nitrogen were found to be a factor, to ascertain why more nitrogen became available in the soil of the spot than in the soil immediately adjacent to it; and (3) to measure the relative yield and quality of grain produced on the spot as compared with that on the field at large. A preliminary report of the 1929 investigations has already been presented.³ The present paper gives in detail the results of the three seasons' studies.

¹ Received for publication, Dec. 28, 1931; issued August, 1932. Contribution No. 144 from the Department of Bacteriology and No. 211 from the Department of Agronomy, Kansas Agricultural Experiment Station.

² LIPMAN, C. B. THE NITRIFYING POWERS OF SOILS AS INDICES TO THEIR FERTILITY. *Soc. Prom. Agr. Sci. Proc.* 35: 73-79. 1915.

³ GAINEY, P. L., and SEWELL, M. C. INDICATIONS THAT AVAILABLE NITROGEN MAY BE A LIMITING FACTOR IN HARD WINTER WHEAT PRODUCTION. *Jour. Amer. Soc. Agron.* 22: 639-641. 1930.

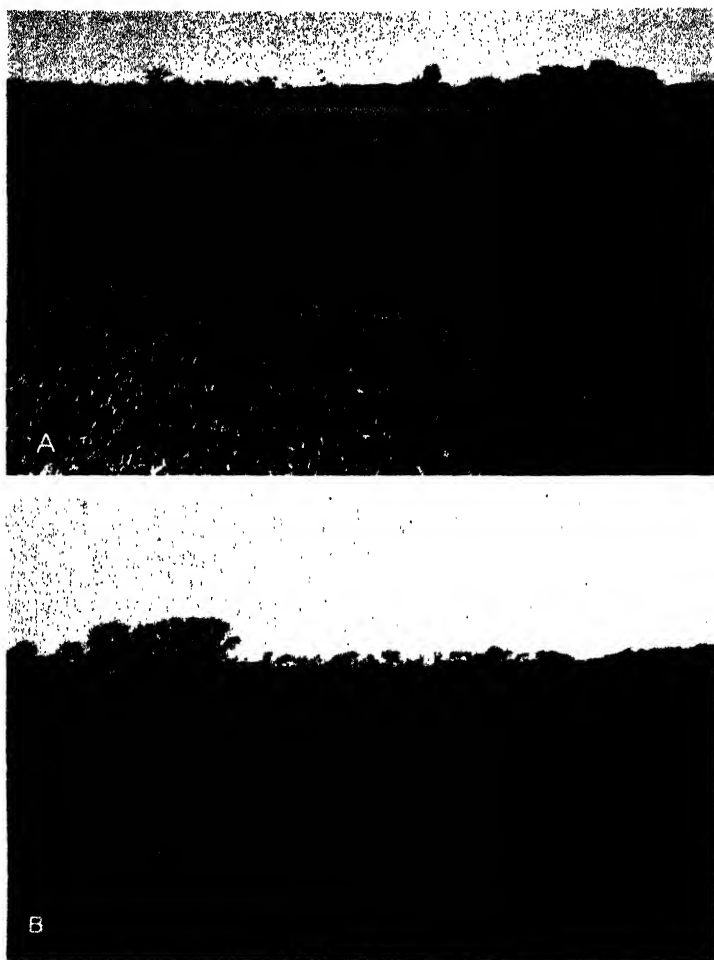


FIGURE 1.—Distant (A) and close (B) views of a typically spotted wheat field

METHODS

For these studies plant material and soil were collected from typical spots and from the area immediately adjacent to them (always within a radius of 10 feet). As Figure 2 indicates, the samples were taken from many fields over a wide territory. In all, 121 samples from 38 counties were collected and studied.

The samples taken from the spots are designated as G (good) and those taken from the field at large as P (poor). The exact technic followed during the three seasons varied somewhat; hence that employed in each year's work is described separately.

NITROGEN STUDIES

DATA FOR 1929

Most of the samples studied in 1929 were collected at some distance from Manhattan; hence laboratory studies could not be made for several days. This necessitated, in the case of soil to be analyzed for nitrate nitrogen, some treatment to prevent any change in the nitrate content during the interval between sampling and analysis. At the same time, it was desired to make certain bacteriological tests. These could not be conducted upon samples of soil to which a preservative had been added. To overcome these difficulties, two samples of soil were collected from a typical spot and two from the area immediately surrounding it. If the soil was dry enough to be handled with a soil tube, one was used, otherwise a garden trowel was employed. In either case the sample was a composite of a number of smaller samples, usually not less than six, taken to a depth of approximately 7 inches. The soil was placed in pasteboard cartons, and to one of the samples

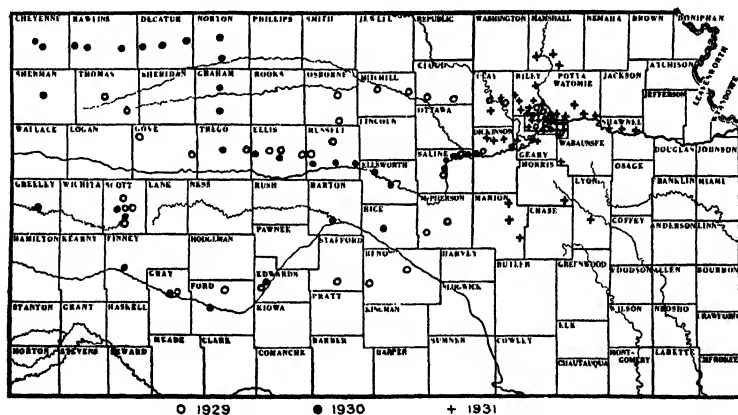


FIGURE 2.—Locations from which samples of plant material and soil were collected

a heavy application of toluol was added and mixed thoroughly with the soil. As soon as this sample reached the laboratory it was analyzed for nitrate nitrogen by the phenol-disulphonic acid method. The sample was then dried, ground, and analyzed for total nitrogen by the Kjeldahl method.

The sample that received no toluol was used for bacteriological studies. It was first passed through a coarse sieve, three meshes per centimeter, and the moisture content and water-holding capacity were then determined. Duplicate samples, the equivalent of 100 g of dry soil, were weighted into wide-mouth cotton-stoppered bottles and sufficient water added to bring the moisture content up to two-thirds saturation. These samples were held at room temperature for six weeks, the moisture lost through evaporation being replaced weekly. After six weeks' incubation the nitrate-nitrogen content was determined.

No special effort was made either in 1929 or the succeeding years to eliminate contamination completely. It was felt that, since there

was every opportunity for the transfer of soil from a spot to the surrounding area, or vice versa, under field conditions, the use of absolutely aseptic conditions would be a waste of effort. However, the instruments used in the field were freed of soil in passing from one sample to another, and in the laboratory the ordinary precautions were observed to prevent the gross transfer of material from one sample to another.

Soil samples were taken from 38 fields in 19 counties. From the same spot and surrounding area from which soil samples were taken, samples of the growing grain were also collected by pulling, thus including the crown and such roots as adhered. In 1929 the plants were not collected from a definite area as in 1930 and 1931. However, the number of plants was recorded, and comparisons are made on the basis of the individual plant. Such values give no indication as to the actual quantity of nitrogen removed from a unit area of soil, but since there was no marked difference in the stand of wheat on the spot and elsewhere, they do give relative values, or the comparison that is of special interest. After the soil had been washed from the roots and the number of plants recorded the entire batch of material was dried, ground, and analyzed for total nitrogen.⁴

The results of the 1929 studies are recorded in Table 1. The data here presented may be briefly summarized as follows:

TABLE 1.—Data obtained from study of plants and soil taken from spots and from surrounding areas—1929

Sample No.	Height of plants		Weight of plants		N in plants		N in soil		NO ₃ in soil		NO ₃ in incubated soil		NO ₃ formed in incubated soil	
	P	G	P	G	P	G	P	G	P	G	P	G	P	G
	<i>Ins.</i>	<i>Ins.</i>	<i>Gms.</i>	<i>Gms.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P. ct.</i>	<i>P.p.m.</i>	<i>P.p.m.</i>	<i>P.p.m.</i>	<i>P.p.m.</i>	<i>P.p.m.</i>	<i>P.p.m.</i>
1.....	6	28	1.48	3.76	1.21	2.38	.1360	.2020	9.7	17.5	88	206	78.3	248.5
2.....	9	28	2.01	5.38	1.00	1.85	.2160	.2180	9.2	8.5	175	313	165.8	304.5
3.....	5	21	1.68	3.05	.88	1.17	.0560	.0605	9.0	18.0	80	186	71.0	168.0
4.....	5	21	.50	2.67	1.06	1.74	.0760	.0810	8.7	8.5	63	105	54.3	96.5
5.....	6	15	.80	2.91	.98	2.31	.1025	.1035	7.5	9.0	82	110	74.5	101.0
6.....	10	27	1.08	5.67	1.73	2.24	.1030	.0990	20.2	26.7	80	104	69.8	77.3
7.....	5	18	.20	1.47	1.39	2.61	.1655	.1950	22.7	31.0	187	568	164.3	537.0
8.....	18	33	1.74	4.59	1.05	2.43	.1080	.1100	19.8	28.5	86	146	66.2	117.5
9.....	12	26	1.58	5.93	1.07	1.89	.1290	.1395	19.2	27.8	108	240	88.8	212.2
10.....	14	26	1.62	7.39	1.20	2.29	.1410	.1765	19.2	16.9	100	426	80.8	409.1
11.....	7	12	.27	.53	1.49	2.81	.1135	.1380	18.5	73.6	117	1,060	98.5	986.4
12.....	10	20	2.33	4.44	.82	2.15	.0330	.0360	17.0	120.7	61	114	44.0	-6.7
13.....	8	16	.25	.88	1.64	2.21	.1100	.1140	17.7	26.2	150	180	132.3	153.8
14.....	6	12	.60	.73	1.38	2.82	.1145	.1360	16.3	42.3	147	820	130.7	777.7
15.....	10	16	2.12	2.10	1.17	1.96	.1415	.1530	16.8	58.3	169	382	152.2	323.7
16.....	8	16	1.42	4.90	1.38	1.86	.1590	.1660	14.7	60.8	200	432	185.3	371.2
17.....	7	12	.44	.72	1.47	2.57	.1380	.1410	24.4	60.1	157	273	132.6	212.9
18.....	6	10	.33	.51	1.75	3.03	.1080	.1250	18.1	34.4	113	696	94.9	631.6
19.....	4	5	.28	.51	2.06	3.97	.1160	.1485	19.1	163.5	96	625	76.9	461.5
20.....	5	11	.09	.49	1.91	2.84	.1380	.1390	18.5	157.0	120	253	101.5	96.0
21.....	6	8	.31	.54	1.99	3.35	.1425	.1415	17.4	21.0	212	259	194.6	238.0
22.....	5	8	.32	.63	2.10	3.86	.1500	.1500	18.3	35.5	167	560	148.7	524.2
23.....	5	7	.22	.31	2.01	3.62	.1325	.1260	19.1	72.4	164	388	144.9	315.6
24.....	10	15	7.2	1.18	1.43	2.87	.1730	.1865	18.0	58.4	165	840	147.0	781.6
25.....	11	17	1.92	5.58	1.65	2.50	.1405	.1480	18.3	73.2	156	152	137.7	78.8
26.....	10	16	1.52	2.53	1.30	2.69	.1405	.1580	15.9	23.2	114	802	98.1	778.8
27.....	10	14	.44	.49	1.53	2.65	.1685	.1885	16.2	67.9	286	498	269.8	430.1
28.....	8	16	.74	1.26	1.49	2.21	.1285	.1375	17.8	22.4	266	222	248.2	199.6
29.....	12	18	1.06	3.05	1.34	2.69	.1490	.1870	20.9	21.9	240	1,440	219.1	1,418.1
30.....	12	18	1.14	2.65	1.31	2.46	.1105	.1195	33.9	35.8	166	481	132.1	445.2
31.....	8	11	.40	.89	1.85	3.45	.1855	.2210	15.8	35.4	248	1,116	232.2	1,080.6
32.....	11	20	.59	1.36	1.26	2.94	.1605	.1780	118.5	166.5	280	1,346	141.5	1,199.5
33.....	9	20	.61	1.42	1.34	2.17	.1120	.1385	17.3	65.0	166	445	148.7	380.0
34.....	6	12	.24	.35	1.52	2.99	.1385	.1630	22.5	29.4	161	1,600	138.5	1,570.6
35.....	9	18	.28	1.04	1.38	3.01	.1235	.1510	20.6	73.7	172	1,200	151.4	1,126.3
36.....	8	14	.61	1.14	1.68	3.47	.1395	.1445	18.4	137.6	360	200	341.6	62.4
37.....	9	19	.47	2.57	1.45	2.53	.1455	.1565	18.0	81.7	158	400	120.0	368.3
38.....	10	24	.45	2.62	.99	2.40	.0960	.1075	16.1	48.1	156	453	141.9	403.9
Av.....	8.4	17.1	.87	2.32	1.43	2.63	.1289	.1434	20.2	52.4	158	518	137.4	465.3

⁴ The total nitrogen determinations on both soil and plant materials for 1929 and 1930 were made in the analytical laboratory of the Department of Chemistry under the supervision of Prof. W. L. Latschaw.

The average height of the G plants was 17.1 inches and of the P plants 8.4 inches, the former being taller than the latter in every comparison. The average weight of the G plants was 2.32 and of the P plants 0.87 g, there being only one instance in which the average weight of the P plant exceeded that of the G. In several instances, the exception just noted being one, there was an excessive number of plants within the spot, resulting in the absence of stooling and the development of slender, weak plants; otherwise the differences in weight between the P and G plants would have been even more marked.

The percentage of nitrogen in the P plants ranged from 0.82 to 2.10, with an average of 1.43, while the corresponding values for the G plants ranged from 1.74 to 3.97, with an average of 2.63. Without exception, the nitrogen content of the G sample exceeded that of the P sample. Since the average weight of the G plants was 2.67 times that of the P plants and the average nitrogen content of the former was 1.84 times that of the latter, the average quantity of nitrogen removed from the soil occupied by a G plant was 4.91 times that removed from the corresponding area occupied by a P plant.

Even though the G plants had removed 4.91 times as much nitrogen from the soil as had the P plants, the G soil when collected still contained, on an average, approximately 2.6 times as much nitrate nitrogen as did the P soil, the average NO_3 content being 52.4 and 20.2 p. p. m., respectively.

When the moisture content of the P and G soils was made up to optimum and the soils placed under identical incubation conditions for six weeks, the quantity of NO_3 present showed a marked increase in practically every sample and the quantity found in the G sample exceeded that in the P sample in 35 of the 38 comparisons, the average for P being 158 and for G, 518 p. p. m. If the NO_3 present originally be deducted from that present at the end of the six weeks' incubation, the quantity formed during the incubation period can be approximated. The average for the P samples is then found to be 137.4 and for the G samples 465.3 p. p. m. Obviously, the rate of accumulation of NO_3 in the G soil was relatively greater when the two were placed under identical conditions than it was in the field, for while the original NO_3 content of the G soil was 2.59 times that of the P soil, the accumulation of NO_3 in the G soil in the laboratory was 3.45 times that in the P soil. The difference in the accumulation under the two conditions can be partly explained by the more rapid absorption of NO_3 from the G soil under field conditions as a result of the heavier plant growth.

As would be expected in a group of soils collected from such widely scattered areas, there was a very marked variation in the nitrogen content of the different samples. A rather unexpected difference noted, however, was the uniformly higher nitrogen content of the G sample as compared with the P, there being only four exceptions to this rule. In many instances, the differences were not very great but the average was significant, being for the P and G soils 0.1289 and 0.1434, respectively, or 11 per cent higher for the G than for the P soils.

If the 38 comparisons are arranged in the order of the magnitude of the difference in nitrogen content between the P and G samples,

as has been done in Table 2, it is quite evident that, in spite of a number of marked exceptions, the excessive accumulation of NO_3 in G over P is definitely associated with the excess of nitrogen in G.

TABLE 2.—*Relationship between excess nitrogen in soil from spots and excess NO_3 accumulation (data from Tables 1 and 4), the comparisons being arranged in order of magnitude*

Sample No.	1929 data ^a		Sample No.	1931 data ^b	
	Excess N in G soil ^c	Excess NO_3 accumulation in G soil		Excess N in G soil ^c	Excess NO_3 accumulation in G soil
	Pounds per acre	P. p. m.		Pounds per acre	P. p. m.
1.....	1,340	170	20.....	654	926
29.....	760	1,199	32.....	608	524
10.....	710	328	5.....	516	664
31.....	710	848	37.....	496	611
19.....	650	385	31.....	474	540
7.....	590	373	18.....	420	261
34.....	590	1,432	39.....	382	325
35.....	550	975	16.....	376	537
33.....	530	231	19.....	370	180
11.....	490	888	2.....	368	78
14.....	430	647	22.....	308	224
27.....	400	160	7.....	348	328
26.....	350	681	33.....	292	421
32.....	350	1,058	8.....	284	17
18.....	340	537	10.....	284	371
24.....	270	635	25.....	264	157
15.....	230	172	27.....	264	100
38.....	230	262	9.....	224	183
37.....	220	248	13.....	216	191
9.....	210	123	4.....	206	140
28.....	180	-49	17.....	158	140
30.....	180	313	28.....	158	201
25.....	150	-59	29.....	146	64
16.....	140	186	11.....	142	-13
4.....	100	42	12.....	134	125
36.....	100	-279	34.....	128	95
20.....	100	-6	23.....	120	169
3.....	90	97	35.....	106	273
13.....	80	22	42.....	104	11
12.....	60	-51	21.....	98	6
17.....	60	80	14.....	82	30
2.....	40	139	40.....	82	114
8.....	40	51	6.....	60	63
5.....	20	27	26.....	60	-3
21.....	-20	43	43.....	50	35
22.....	-50	376	36.....	38	51
6.....	-80	8	3.....	38	63
23.....	-130	171	15.....	30	32
			24.....	22	57
			41.....	8	72
			1.....	-30	30
			30.....	-38	-8

^a Correlation coefficient, 1929, 0.527 ± 0.070 .

^b Correlation coefficient, 1931, 0.843 ± 0.030 .

^c 2,000,000 pounds of soil.

The low correlation coefficient (0.527 ± 0.079), together with the fact that in all four instances in which the nitrogen content of P exceeded that of G the latter still exhibited a much higher NO_3 accumulation, indicates very strongly that some factor other than total nitrogen was influencing the rate of nitrate accumulation. However, in the 20 instances in which the G soil contained over 200 pounds per acre more nitrogen than did the P soil, the average excess accumulation of NO_3 was 568 p. p. m., ranging from +123 to +1,432 p. p. m.,

while the corresponding values for the 18 comparisons in which the difference in total nitrogen between P and G was less than 200 pounds per acre averaged 62 and ranged from -279 to +376 p. p. m. On the other hand, while the average total nitrogen was only 11 per cent more in the G than in the P soil, the average NO_3 accumulation during the six weeks' period was 239 per cent more in the G soil.

Flasks of Ashby's mannite solution were inoculated with both P and G soil, but the differences between the two soils in the type and quantity of growth that took place were so slight as to indicate that quantitative nitrogen determinations would yield no information of value; hence none were made. Where differences in growth between the P and G samples were evident they were, in most instances, in favor of the P samples.

DATA FOR 1930

During the spring of 1930 samples of soil and growing grain were collected from spots and from the immediately adjacent areas in 40 fields in 21 different counties. The methods used were similar to those employed in 1929, except that no sample of soil was taken for bacteriological studies and the growing grain was collected by cutting it just above the surface of the ground from a definite area, 1 or 2 square feet, depending upon the size and thickness of the plants.

The data for 1930 are presented in Table 3.

TABLE 3.--Data obtained from study of plants and soil taken from spots and from surrounding areas in 1930

Sample No.	Height of plants		Weight of plants		N in plants		N in soil		NO_3 in soil	
	P	G	P	G	P	G	P	G	P	G
	Inches	Inches	Grams	Grams	Per cent	Per cent	Per cent	Per cent	P. p. m.	P. p. m.
1.	26	34	36.5	116.0	1.010	1.295	0.1120	0.1100	10.90	9.26
2.	26	34	28.2	104.0	1.220	1.650	.1400	.1450	11.99	29.72
3.	15	30	23.7	98.7	1.370	2.000	.2040	.2105	15.08	29.94
4.	15	27	29.5	65.7	1.375	1.620	.1565	.1585	52.97	28.76
5.	18	24	16.0		1.520		.1470	.1550	10.60	42.10
6.	24	36	41.0	124.0	1.440	1.745	.1975	.2095	7.88	19.88
7.	18	30	19.0	80.0	1.440	1.950	.1545	.1800	10.98	21.01
8.	16	22	11.0	55.0	1.010	1.830	.1200	.1295	12.63	28.13
9.	18	28	25.0	95.0	1.595	2.160	.1220	.1215	10.24	52.95
10.	12	18	14.0	38.0	1.485	2.435	.1065	.1080	8.67	16.27
11.	14	24	18.0	70.0	1.410	2.475	.0895	.1000	14.26	38.96
12.	18	18	22.0	25.0	1.545	2.580	.0905	.1020	11.05	52.47
13.	15	18	32.0	56.0	1.830	2.425	.1685	.1610	10.63	12.91
14.	15	23	40.0	81.0	1.550	2.990	.1490	.2200	9.51	329.22
15.	10	16	10.0	38.0	1.885	2.750	.1130	.1070	14.01	16.93
16.	16	21	20.0	45.0	1.690	2.715	.1310	.1340	12.54	33.72
17.	15	18	23.0	62.0	1.570	2.685	.1400	.1520	10.21	31.20
18.	12	16	16.0	46.0	1.840	2.970	.2100	.2150	8.52	17.04
19.	6	11	9.0	25.6	2.470	3.440	.0990	.1250	8.49	35.38
20.	11	20	22.0	34.0	2.215	3.270	.1520	.1510	12.22	29.48
21.	12	17	21.0	49.0	1.600	2.920	.1680	.1750	8.48	14.15
22.	18	24	34.0	50.0	1.490	2.530	.1265	.1940	9.29	39.00
23.	12	18	12.0	29.0	1.770	3.040	.1180	.1280	9.41	45.92
24.	12	18	17.0	49.0	1.895	3.265	.1410	.1565	10.09	52.00
25.	8	11	8.5	22.0	2.000	3.705	.1190	.1210	9.61	18.13
26.	13	21	23.0	58.0	1.330	2.970	.1120	.1200	10.04	17.94
27.	10	18	13.0	56.0	1.870	3.055	.1165	.1300	9.86	36.48
28.	18	28	16.0	71.0	1.325	2.165	.1310	.1260	9.75	16.02
29.	13	18	25.0	62.0	1.475	2.755	.1220	.1160	10.00	14.10
30.	15	21	18.0	43.0	1.560	3.180	.1130	.1145	8.67	83.31
31.	17	23	23.0	66.0	1.520	1.940	.1420	.1455	10.21	14.15
32.	16	24	20.0	80.0	1.800	2.770	.1270	.1450	9.55	51.57
33.	20	36	32.0	93.0	1.610	2.130	.1885	.1985	9.51	19.78

TABLE 3.—Data obtained from study of plants and soil taken from spots and from surrounding areas in 1930—Continued

Sample No.	Height of plants		Weight of plants		N in plants		N in soil		NO ₃ in soil	
	P	G	P	G	P	G	P	G	P	G
	<i>Inches</i>	<i>Inches</i>	<i>Grams</i>	<i>Grams</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>P. p. m.</i>	<i>P. p. m.</i>
34.....	20	32	28.0	103.0	1.390	1.770	.1620	.1600	9.41	14.07
35.....	20	28	27.0	69.0	1.330	1.720	.1460	.1455	8.72	33.51
36.....	25	29	59.0	99.0	1.120	1.990	.1200	.1365	7.73	46.55
37.....	18	30	30.0	119.0	1.150	2.080	.1150	.1390	9.14	42.91
38.....	18	20	18.0	43.0	1.700	2.780	.1360	.1610	9.53	33.18
39.....	24	28	29.0	63.0	1.330	2.080	.1555	.1840	8.91	87.73
40.....	18	28	27.0	91.0	1.550	1.900	.1550	.1515	10.00	12.85
Average.....	16.02	23.5	23.41	66.30	1.572	2.457	.1379	.1486	11.297	39.20

In practically every instance the plants were taller and the dry weight greater from the spot than from the area immediately adjacent, the average values being as follows: For height, P 16 inches, G 23.5 inches; for dry weight, P 23.41 g and G 66.30 g. Furthermore, the percentage of nitrogen in the plants from the G soil was invariably greater than that in the plants from the P soil, the latter varying from 1.010 to 2.470 and averaging 1.572, while the former ranged from 1.295 to 3.705 and averaged 2.457. There were only three of the P samples in which the nitrogen content exceeded 1.9 per cent, whereas only seven of the G samples contained less than that amount. In 1929 there were five P samples with a nitrogen content in excess of 1.9 per cent and four G samples with a smaller percentage of nitrogen.

Since the average weight of dry matter per unit area from G was 2.83 times that from P and the nitrogen content of the plant material 1.56 times that of P, the G plants had absorbed 4.41 times as much nitrogen as the P plants growing upon equal areas. The corresponding value for 1929 was 4.91.

The average NO₃ content of the P soil was 11.3 and that of the G soil 39.2 p. p. m., the G exceeding the P in 38 of the 40 comparisons, the average NO₃ content of the G soil being 3.47 times that of the P soil. The corresponding figure for 1929 was 2.6.

The average nitrogen content of the 40 P soils was 0.1379 per cent and of the G soils 0.1485 per cent, as compared with 0.1289 and 0.1434 per cent, respectively, for 1929. No samples were incubated during the 1930 season.

DATA FOR 1931

In 1931, 43 samples were collected from 11 counties. All the samples were taken within a radius of 100 miles of Manhattan, which made it possible to initiate laboratory studies within a few hours after the samples were collected and to utilize the same sample for chemical and bacteriological studies. Nitrate and total nitrogen analyses of the soil were run as in previous years. After moisture content and water-holding capacity were determined, duplicate 100 g (dry basis) samples were weighed into wide-mouth cotton-stoppered bottles, and the moisture was made up to two-thirds saturation and held at room temperature for four weeks, the moisture lost being replaced weekly. Two additional samples were treated in the same way, except that 1 g of cottonseed meal containing 7 per cent nitrogen was thoroughly

mixed into the soil. All four samples were analyzed for nitrate nitrogen after four weeks' incubation.

The nitrogen-fixing ability of the various soils was determined by inoculating (in quadruplicate) poured plates containing 20 c c of carefully washed mannite agar with 1 g of soil, immediately analyzing two for total nitrogen and incubating the remaining two at 28° C. two weeks, after which the nitrogen content was determined. During incubation the plates were examined at frequent intervals and the relative growth of *Azotobacter* recorded.

The growing grain was cut from an area of 2 square feet, dried, weighed, ground, and analyzed for total nitrogen.

The 1931 data are presented in Table 4. In every one of the 36 comparisons there shown the height and dry weight of plants from the spot exceeded that from the surrounding area, the average height being P 11.9 and G 20.7 inches, and average weight P 39.5 and G 114.9 g. The nitrogen content of the P plants ranged from 0.80 to 2.83 per cent and averaged 1.55 per cent, while that of the G material varied from 1.22 to 4.10 per cent and averaged 2.56 per cent. There were only three P samples in which the nitrogen content was higher than 1.9 per cent and only four G samples in which the nitrogen content was lower than that amount.

[illegible]

40	9	18	15.6	85.0	1.61	2.88	.1549	3.0	5.9	77.9	195.2	74.9	198.3	1,536.0	1,402.0	1,438.1	1,206.8	3.89	5.24
41	16	20	20.2	79.7	.80	2.61	.1477	5.5	53.4	78.2	196.8	71.7	143.4	1,145.0	1,093.0	1,096.8	872.2	.04	-.30
42	24	30	27.8	150.5	.92	1.22	.1393	3.7	3.7	61.8	72.8	58.6	69.1	938.1	1,168.0	896.3	1,065.2	-.13	-.30
43	15	20	43.7	76.6	1.17	2.36	.1844	4.1	4.0	104.8	138.6	100.7	135.6	1,145.0	1,128.0	1,040.2	989.4	-.38	-.38
Average	11.9	20.7	39.5	114.9	1.55	2.56	.1447	6.5	47.6	76.3	323.4	69.7	275.8	1,113.6	1,327.9	1,040.9	1,004.5	2.28	2.18

* Cottonseed meal 1 g per 100 g of soil.

Since the average weight of dry plant material from equal areas was 2.91 times as much from the G as from the P soil and the nitrogen content 1.65 times as great in the G soil, there had actually been removed, per unit area, 4.80 times as much nitrogen from the G soil as from the P. The corresponding values for 1929 and 1930 were 4.91 and 4.41, respectively. In spite of the fact that 4.80 times as much nitrogen had been removed from the G as from the P soil, the soil from the spots still contained an average of 47.6 p. p. m. NO_3 as compared with an average of 6.5 p. p. m. in the surrounding soil. There were only 5 of the 43 comparisons in which the NO_3 content of the P soil exceeded that of the G. In one case they were the same.

When the moisture content of the soils was brought to optimum and the two held under identical conditions of temperature, aeration, etc., for four weeks, a very rapid accumulation of NO_3 took place in practically all samples, the accumulation being approximately four times as rapid in the G soil as in the P. The average quantities of NO_3 found after incubation were 76.3 p. p. m. for the P samples and 323.4 for the G samples, the quantities formed during the 4-week period being on an average 69.7 p. p. m. in the the P soil and 275.8 in the G soil. These comparative values, together with the almost identical results obtained in 1929, lead inevitably to the conclusion that there must have been present in the G soil either a much more active nitrifying flora or a quantity of nitrogen capable of being much more easily transformed into the nitrate condition. If the differences in favor of the G soil were due to a more active microflora, then when an abundance of easily transformed nitrogen was added to the two soils in equal quantities the microflora of G should be capable of transforming the added nitrogen into NO_3 at a much more rapid rate, whereas, if no difference existed in the two microfloras, the rate at which the added nitrogen would be transformed into nitrate nitrogen should be approximately the same.

A comparison of the nitrate content of the P and G soils to which cottonseed meal was added shows the NO_3 content of the latter to be appreciably higher than that of the former, the average values being 1,113.6 and 1,327.9 p. p. m., respectively, for P and G. However, if the NO_3 present in the incubated soil not receiving cottonseed meal be subtracted from that present in samples receiving the added nitrogen, then an approximation of the nitrate nitrogen actually formed from the added nitrogen is reached. When this is done it is found that the values are on an average practically identical, being for the P soil 1,040.9 and for the G soil 1,004.5 p. p. m. Furthermore, in 22 of the 42 comparisons the P soil exceeded the G, while in the other 20 the reverse was true. This is almost conclusive evidence that the difference in the accumulation of NO_3 is not connected with a more efficient microflora in the soil of the spots, but is in some way associated with differences in the quantity or quality of the nitrogen content.

Again, as in the two preceding seasons, the average nitrogen content of the G samples was found to be slightly higher than that of the P samples, the actual values being 0.1447 and 0.1555 per cent. In only two comparisons did the nitrogen content of the P sample exceed that of the G.

If the soils are arranged in the order of the magnitude of the difference in nitrogen between the P and G samples, as was done for the

1929 data, and these differences compared with those in nitrate accumulation during the 4-week incubation period, it will be noted that the two are associated. (Table 2.) The coefficient of correlation is 0.843 ± 0.030 , as compared with 0.527 ± 0.079 for 1929, a significant correlation. The association is more strikingly shown if the average NO_3 accumulation of those soils in which the difference in nitrogen content exceeded 200 pounds is compared with that of the soils in which the difference was less marked. In the former group the average excess NO_3 accumulation in G over P is 339 p. p. m., and in the latter only 74 p. p. m., as compared with 568 and 62 for the 1929 data.

In spite of definite indications that the higher nitrate content of the G soil is associated with the higher total nitrogen content, the large number of instances, both in the 1929 and 1931 data, in which P samples with a relatively high total nitrogen content showed relatively low NO_3 accumulation, and G samples with relatively low total nitrogen content showed high NO_3 accumulation, indicate that the total nitrogen alone can not account for the difference in the rate of nitrate accumulation. There are comparatively few instances in which the maximum accumulation of NO_3 in the P soil, regardless of the total nitrogen content, has equaled the minimum accumulation in the G samples. A concrete illustration will suffice to emphasize this point. Samples 34 and 32 contained the lowest percentage of nitrogen of any of the G soils in the 1931 series, both being lower than any P sample except their corresponding numbers, yet the accumulation of NO_3 in the 34 G sample was exceeded by that in only 1 P sample, and the accumulation of NO_3 in the 32 G sample was 3.5 times the highest accumulation in any P sample.

That there must be a difference in the quality as well as the quantity of the nitrogen present in the G soil as compared with that in the P soil is further substantiated by the following comparison: On an average, the G soil contained only 7.5 per cent more nitrogen than the P soil, yet during the 4-week incubation period the G soil formed 296 per cent more NO_3 . The corresponding values for the 1929 data were 11 and 239 per cent.

A second attempt was made in 1931 to measure the relative nitrogen-fixing ability of the soil from spots and from the soil immediately surrounding them. Poured plates of carefully washed mannite agar were inoculated with a definite quantity of soil, incubated for two weeks, and the quantity of nitrogen determined. Colony counts were also made at frequent intervals. On the whole, no significant difference was found in the quantity of nitrogen fixed, the average for the 43 comparisons being 2.28 and 2.18 mg per plate for the P and G samples, respectively. In a few instances significant differences were observed, the difference being sometimes in favor of one sample and sometimes in favor of the other. There was, however, a rather striking difference between the two samples in the early development of *Azotobacter* colonies. Approximately one-half the soils failed to show appreciable development of the bacteria, *Azotobacter* being either completely absent or confined to a few isolated colonies, possibly arising from contaminations. In a few instances the development of colonies was quite similar in both samples, but in most cases there was a striking difference in favor of the P soil. After *Azotobacter* had grown for two weeks, the total mass was approximately

equal in the two samples; hence no marked difference in nitrogen fixation was evident. The reason for the presence of larger numbers of *Azotobacter* in the P soil is not clear. It is possible that the growth of *Azotobacter* in the presence of an abundance of available nitrogen, such as occurred in the soil of the G spots, resulted in a temporary lowering or loss of nitrogen-fixing ability and that when the bacteria were transferred to a pabulum practically devoid of combined nitrogen many were unable to survive. However, those that did survive soon regained their nitrogen-fixing ability and thrived.

YIELD STUDIES

DATA FOR 1929

Because of the wide distribution of fields from which soil samples were studied and the very short time in which samples of mature grain could be collected, it was impossible to obtain yield data from the same fields from which the soil samples were taken. Comparisons of yield have been confined to four counties, Riley, Clay, Dickinson, and Geary. For the season of 1929 samples of ripe grain were harvested from spots and the area immediately adjacent in 20 wheat and 2 oat fields. The same four drill rows were cut for a distance of 2 feet in the spot and immediately adjacent to the spot. In some instances the cutting was actually continuous, and in no instance was it separated by more than 2 feet. The harvested area approximated 4 square feet, or 0.0001 acre. The 1929 data are recorded in Table 5.

TABLE 5.—Yield of wheat or oat grain from equal areas of 4 square feet, P and G soil, in 1929

Sample No.	Yield (grams) of grain on—		Sample No.	Yield (grams) of grain on—		Sample No.	Yield (grams) of grain on—	
	P area	G area		P area	G area		P area	G area
1.....	48.45	97.47	9.....	51.36	115.46	17.....	50.13	108.44
2.....	35.56	117.91	10.....	36.65	144.69	18.....	50.80	143.65
3.....	42.38	108.59	11.....	55.71	110.14	19.....	51.12	108.10
4.....	55.59	138.38	12.....	45.94	123.65	20.....	66.85	167.54
5.....	28.14	165.49	13.....	44.07	96.38	21.....	50.28	110.52
6.....	30.82	146.52	14.....	17.51	77.62	22.....	52.06	222.44
7.....	50.25	138.88	15.....	16.56	146.17			
8.....	43.35	96.10	16.....	47.23	121.79	Average.....	44.13	127.54

^a Oats.

^b 10.89 per cent protein in composite sample, oat samples not included.

^c 12.37 per cent protein in composite sample.

The average yield of wheat and oats from the P areas was 44.13 g and from the G areas 127.54 g, the latter being 2.89 times the former. A composite of the wheat samples was analyzed for total nitrogen and found to contain 1.91 and 2.17 per cent, respectively, for the P and G soils, or 10.89 and 12.37 per cent protein.

DATA FOR 1931

In 1931 samples of mature grain were harvested, in the same manner as in 1929, from P and G areas in 27 fields. The weight of grain, percentage of nitrogen in each sample, and percentage of protein calculated from the nitrogen are presented in Table 6. The essential points to be noted from the data in this table are that the yield of

grain from the G areas average 2.12 times that from equal P area, and contained an average of 29.19 per cent increase in protein content. The corresponding values for 1929 were 2.89 and 13.59, respectively. In only 2 of the 27 comparisons did the grain from the P area contain as much nitrogen as that from the G area, while the maximum excess nitrogen in grain from a G area over that in grain from a P area was 96 per cent.

TABLE 6.—Yield and protein content of wheat grain from equal areas, 2 square feet, of P and G soil in 1931

Sample No.	Weight of grain		Nitrogen in grain		Protein content of grain		Increase or decrease in protein in grain from G soil over that in grain from P soil *
	P	G	P	G	P	G	
	Grams	Grams	Per cent	Per cent	Per cent	Per cent	Per cent
1	33.0	122.5					
2	108.0	228.0	1.85	2.01	10.55	11.46	8.63
3	66.5	212.0	1.67	2.19	9.52	12.48	31.09
4	93.0	143.0	1.85	2.19	10.55	12.48	18.29
5	53.0	150.5	1.63	2.22	9.29	12.65	36.16
6	43.0	102.0	1.86	2.42	10.00	13.79	30.09
7	25.0	84.0	2.12	2.36	12.08	13.45	11.34
8	57.0	127.0	1.73	2.12	9.86	12.08	22.52
9	89.0	169.0	1.78	2.39	10.15	13.62	34.19
10	96.0	147.5	1.76	2.53	10.03	14.42	43.77
11	61.0	182.0	1.73	2.28	9.86	13.00	31.85
12	40.5	132.0	1.30	2.30	7.40	13.11	77.16
13	82.0	117.0	1.68	2.25	9.58	12.83	33.92
14	87.0	119.0	1.43	1.72	8.15	9.80	20.25
15	64.0	112.0	1.25	2.45	7.13	13.96	95.79
16	49.5	114.0	1.87	2.01	10.06	11.46	7.80
17	26.0	63.0	1.87	2.50	10.06	14.25	33.68
18	65.5	119.5	1.70	2.46	9.69	14.02	44.68
19	81.5	166.0	1.60	2.64	9.12	15.05	65.02
20	80.0	161.5	1.85	1.79	10.54	10.20	-3.23
21	43.0	113.0	1.90	2.30	10.83	13.11	21.05
22	53.0	132.0	1.92	2.00	10.94	11.40	+4.20
23	59.0	101.0	1.76	2.46	10.03	14.02	39.78
24	52.0	110.0	1.94	2.37	11.06	13.51	22.15
25	65.0	95.5	2.24	3.68	12.77	20.98	64.20
26	52.0	104.0	2.12	2.55	12.08	14.54	20.36
27	47.0	109.0	2.62	2.59	14.93	14.76	-1.14
Average	61.9	131.0	1.81	2.34	10.31	13.32	29.19

* Percentage increase or decrease in percentage of protein.

DISCUSSION

From a determination of the quantity of growth on equal areas within and without typical spots and an analysis of the plant material for total nitrogen, information was obtained relative to the actual quantities of nitrogen utilized under the two conditions. Since the quantity of a particular plant-food element available in the soil may influence the percentage of that element in the plant, as well as the total quantity absorbed, the quantitative analysis of the plant material was doubly important. As a result of these determinations it was found that, with one possible exception, the dry weight of plant material produced per unit area in the 113 comparisons was greater in the spot than in the area immediately adjacent (Table 7), the average values of G for the three years being, respectively, 2.67, 2.83, and 2.91 times those of P.

TABLE 7.—Summary of data obtained in 1929, 1930, and 1931

Item	Average height of plant		Average weight of plant material		Average nitrogen in plants		Average nitrogen in soil		Average NO ₃ in soil	
	P	G	P	G	P	G	P	G	P	G
	<i>In.</i>	<i>In.</i>	<i>Grams</i>	<i>Grams</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>P. p. m.</i>	<i>P. p. m.</i>
1929 ^a	8.4	17.1	0.87	2.32	1.43	2.63	0.1289	0.1434	20.2	52.4
1930 ^b	16.0	23.5	23.41	66.30	1.57	2.46	.1379	.1485	11.3	39.2
1931 ^c	11.9	20.7	39.50	114.90	1.55	2.56	.1447	.1555	6.5	47.6
Total comparisons, number	114		113		113		121		121	
Pairs in which G exceeded P, number	113		112		112		104		111	
Comparisons in which G exceeded P, per cent	99		99		99		86		92	

Item	Average NO ₃ in incubated soil		Average NO ₃ formed during incubation		Average NO ₃ in incubated soil containing cottonseed meal		Average NO ₃ formed from cottonseed meal during incubation		Average nitrogen fixed, per plate	
	P	G	P	G	P	G	P	G	P	G
	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>P. p. m.</i>	<i>Milli-grams</i>	<i>Milli-grams</i>
1929 ^a	158.0	518.0	137.4	465.3						
1930 ^b										
1931 ^c	76.3	323.4	69.7	275.8	1,113.6	1,327.9	1,040.9	1,004.5	2.28	2.18
Total comparisons, number	80		80		43		42		43	
Pairs in which G exceeded P, number	75		72		30		20		20	
Comparisons in which G exceeded P, per cent	94		90		70		+48		47	

^a Weight of plant material based upon individual plant.^b Weight of plant material from 1 square foot.^c Weight of plant material from 2 square feet.

In the 113 comparisons the percentage of nitrogen found in the G material was greater than that in the P material in all instances except one, the actual average percentage of nitrogen being 1.84, 1.57, and 1.65 times as great in G as in P. The calculated quantity of nitrogen absorbed from the soil per unit area was, at the time the plant material was collected, 4.91, 4.41, and 4.80 times as great for G as for P for the three years under study. These same differences were carried through the grain to maturity, for the total quantity of nitrogen found in the mature threshed grain from the G area exceeded that from the P area by 3.29 and 2.73 times, respectively, for 1929 and 1931.

The above values indicate that more nitrogen was available in the soil of the spot than in the adjacent soil. The quantitative determination of the nitrate nitrogen actually present in the soil gave additional information as to available nitrogen, at least at the time the samples were taken. These data show that even though four and a half times as much nitrogen had been removed from the G soil by the growing plants as from the P soil, there was still present in the G soil, for the three years under study, averages of 2.59, 3.47, and 7.32 times as much NO₃ as was present in the P soil. Furthermore, of the 121 comparisons, there were only 10 in which the P sample contained more NO₃ than the G sample, and the difference in favor of the P sample was significant in only two instances. (Table 7.)

The relatively low NO_3 content of the P soil, averaging only 6.5 p. p. m. for the 43 samples collected in 1931, together with the low nitrogen content of plant material and the absence of dark-green color, indicated that the plants growing therein were maintaining the nitrate level very near the minimum at which absorption can take place, whereas in most of the G soils there was a surplus of available nitrogen.

These data show beyond doubt that more nitrogen was being rendered available for plant metabolism in the soil of the spots than in the soil immediately surrounding them. The next question that arises is, What factor or factors are responsible for this apparently abnormal condition? Seemingly, any one or any combination of three possible conditions might result in the formation of more available nitrogen in the soil of the spots: (1) The microflora of the spots might be more efficient in transforming nonavailable nitrogen into an available form; (2) the environmental conditions of the microflora of the spots under field conditions might be more favorable; or (3) there might be present in the soil of the spots more nitrogen capable of being readily transformed into an available form.

Almost conclusive evidence that the efficiency of the microflora of the two samples of soil were not significantly different is furnished by the 1931 data secured from the samples to which cottonseed meal was added and which were subsequently incubated. In the presence of an excess of organic nitrogen capable of being readily transformed into the nitrate condition the soil with the most active or efficient microflora should accumulate NO_3 most rapidly. Under such conditions it was found that the average transformation of cottonseed-meal nitrogen into nitrate nitrogen was at the rate of 1,004.5 and 1,040.9 p. p. m., respectively, for the G and P samples. There are certainly no indications in these data that the microflora of P was in any way less efficient than that of G.

That the greater quantity of available nitrogen in the spots is not primarily due to environmental conditions is obvious from the fact that in samples of P and G soil incubated under identical conditions of moisture, temperature, aeration, etc., the differences in nitrate-nitrogen content in favor of the G soil were not only maintained but in many instances were actually accentuated (the initial NO_3 content of G being 2.59 times that of P in the 1929 series, while the NO_3 formed in G during six weeks' incubation was 3.39 times that formed in P).

This leaves as the only plausible explanation a difference in the nitrogen content of the soils, either quantitative or qualitative. The quantitative total nitrogen analyses of the soil lend considerable support to this explanation. Of the 121 comparisons between the nitrogen content of P and G, the latter contained a higher percentage of nitrogen in 104 instances, the average difference in favor of G being 0.0145, 0.0107, and 0.0108 per cent, respectively, for the three years. Evidence that the difference in the relative ability of the two samples to accumulate NO_3 is associated with this difference in total nitrogen content is presented in Table 5. However, the low correlation coefficient for 1929, together with the uniformly low NO_3 accumulation in the P and high accumulation in the G samples regardless of the total nitrogen content, as well as the absence of a direct quantitative relationship between the excess total nitrogen and increased NO_3 ,

accumulation (see next to last paragraph, p.141), points conclusively to a qualitative as well as a quantitative difference in the nitrogen content of the soils.

Field observations have led to the conclusion that the greater number, though not all, of the sort of spots under study follow the deposition of urine by grazing cattle. Cow urine, of course, contains nitrogen in the form of urea which can be readily transformed into nitrate nitrogen. If the deductions made in the preceding paragraphs are correct, it should be possible to duplicate these results experimentally. Experiments were carried out in 1929-30 and 1930-31 to see if this were possible. Certain of the data obtained are presented in Tables 8 and 9. The plots were 10 by 20 feet in size. The yields on the check plots for 1930-31 indicate that nitrogen, at least when applied alone, was not a limiting factor in the soil on which the plots for that year were located. It should be noted, however, that the applications of nitrogen, particularly urine, resulted in the production of plots to all appearances similar to spots, but maturity was slightly delayed and such plots were much more seriously injured by a severe late drought. From these experiments it is evident that the essential characteristics of typical naturally occurring spots, i. e., increased growth accompanied by dark-green color, increased NO_3 content of soil, increased yield, and increased protein content of the grain—can be produced experimentally by the application of cow urine and some other forms of nitrogen.

TABLE 8.—Data from experimentally produced spots, 1930

Treatment	Height of plants	Weight of plants	Nitrogen in plant material	NO_3 in soil
	Inches	Grams	Per cent	P. p. m.
Check	14.0	19.6	1.440	12.39
Ca(CN) ₂	20.0	34.1	1.515	9.48
CO(NH ₂) ₂	20.0	40.5	1.560	28.66
Urine	19.0	33.9	2.270	57.76
Check	14.0	16.2	1.540	20.21

TABLE 9.—Yield and protein content of grain from experimentally produced spots

Treatment	1929-30		1930-31	
	Yield per acre	Protein in grain	Yield per acre	Protein in grain
	Bushels	Per cent	Bushels	Per cent
Check	* 14.5	10.5	22.9	11.65
Urine in August, ¼ inch	20.5	12.1	18.9	15.05
Urea in August, 110 pounds per acre	19.1	10.7	24.9	14.55
Check	8.9	11.1	23.4	11.85
Cyanamid in August, 220 pounds per acre	15.6	10.2	28.7	13.50
Urine in March, ¼ inch	18.3	16.6	28.6	15.75
Check	9.5	11.2	26.4	11.35
Urea in March, 110 pounds per acre	19.9	10.4	28.4	13.65
Cyanamid in March, 220 pounds per acre	19.3	9.8	28.5	13.25
Check	7.5	10.9	24.6	11.85
Urea, 55 pounds in August, 55 pounds in March	20.6	9.6	22.0	14.25
Cyanamid, 110 pounds in August, 110 pounds in March	18.5	9.8	23.2	14.00
Check	10.5	10.6	25.5	10.55
Superphosphate (20 per cent), 300 pounds per acre	10.5	10.6		
Potassium chloride, equivalent K in urine	11.1	10.4		
Urea at seeding, equivalent N in urine	25.6	13.9		
Amophos (11-46-0),* 100 pounds at seeding			35.6	13.05
Cyanamid, 200 pounds at seeding			28.6	12.05
Cyanamid, 400 pounds at seeding			26.9	14.50

* Yield abnormally high, plot adjacent to road and subject to drainage and blowing from road.

† Eleven per cent nitrogen, 46 per cent P_2O_5 , 0 per cent potassium.

Of major interest from a practical point of view is the effect of such spots on the yield and quality of the grain. Inquiry among farmers as to the yield on such spots elicited a variety of answers, which may be summed up in the statement that ordinarily the spots outyield the field, but in occasional years the grain of the spots does not fill out, and hence the yield is small. The writers' observations bear this out. The only years for which yield data are available for naturally occurring spots are 1929 and 1931, when the yield from the spots was, respectively, 2.89 and 2.12 times that from the adjacent area. Data for experimentally produced spots in 1930 show differences in yield of the same magnitude. (Table 9.) The years in which spots fail to outyield the adjacent areas are probably associated with one of two circumstances: (1) Conditions so favorable for NO_3 formation in soil that the field at large is supplied an adequate quantity of nitrate nitrogen for maximum yield; or (2) conditions of moisture such that the quantity available late in the season is inadequate for the excessive demands of the heavier growth on the spots.

Of almost equal importance with the increased yield is the marked increased protein content of the grain, both from the naturally occurring and from the experimentally produced spots. The effect of this additional available nitrogen upon the composition of the grain is evident even in the 1931 experimental spots, where questionable increases in yield were recorded.

Because lodging of grain frequently occurs on soils that contain excessive quantities of nitrogen, it is commonly believed that excessive nitrogen is deleterious. In many instances no doubt it is, but during the past three years such has not been found to be the case in the type of spot under study. In order to secure concrete evidence as to the effect that the spots produce on the strength of the straw, breaking tests of the 22 samples of straw harvested for yield in 1929 were conducted with the apparatus constructed by Salmon.⁵ Ten separate tests, comprising 10 straws per test, were carried out on each sample. With one exception, the average breaking strength of the G samples was greater than that of the P samples, the average resistance to breaking being 37.5 per cent greater in the case of G.

Thus far the writers' studies have been confined entirely to nitrogen for the reason that this investigation is a part of the general nitrogen studies under way at this station. No evidence is as yet available to show that other elements may not have had some part in the production of the spots. However, applications of potassium under central and western Kansas conditions have been without effect upon the yield of wheat. Applications of phosphorus do affect yields, particularly in eastern Kansas. However, the thesis upon which this work was based is that the spots arise primarily from cow urine, which contains only a trace of phosphorus. Furthermore, the phosphorus content of the plants taken from the P and G areas in 11 selected fields was found to average, respectively, 0.283 and 0.269 per cent. Neither phosphorus nor potassium experimentally applied alone, the latter in the same concentration in which it occurs in urine, gave any indication of producing characteristic spots. (Table

⁵ SALMON, S. C. AN INSTRUMENT FOR DETERMINING THE BREAKING STRENGTH OF STRAW, AND A PRELIMINARY REPORT ON THE RELATION BETWEEN BREAKING STRENGTH AND LODGING. *Jour. Agr. Research* 43: 73-82, illus. 1931.

9.) The effect of both these nutritive elements is being studied further.

No effort has been made to correlate the occurrence of the type of spots under study with agricultural practices such as methods and time of seed-bed preparation. However, early and thorough preparation are known to be essential to an accumulation of adequate nitrate nitrogen for good wheat yields. The very frequent observation that spots occur on low, heavy soils potentially rich in nitrogen—i. e., soils containing 0.15 to 0.20 per cent, many of which are poorly prepared—emphasizes the necessity of such preparation of the seed bed as will enable the nitrifying organisms to function properly if maximum yields are to be expected. On the other hand, the data given herein relative to the nitrogen content of Kansas soils (the most comprehensive thus far presented) show that efforts are being made to grow wheat upon many soils that are relatively deficient in nitrogen. It is upon such soils that the most striking spots are observed. Continuous cropping to nonleguminous crops will continue to reduce the nitrogen level until an equilibrium is reached,⁶ and it is logical to expect the spotted condition to become more prevalent as time goes on. The data in Table 9, together with much unpublished data, indicate that on many soils the use of commercial nitrogenous fertilizers will, under certain conditions, result in marked increases in yield, the conditions being a low concentration of available nitrogen, unaccompanied by a deficiency in some other nutritive element. The response to such treatment may be expected to increase in the future.

SUMMARY

This paper presents the results of three years' study of the rôle of nitrogen in the production of the type of spot frequently observed in small-grain fields in Kansas. These spots are almost invariably accompanied by a higher total nitrogen content of the soil, higher NO_3 content of the soil, higher percentage nitrogen composition of the growing plant, higher total nitrogen absorption, higher yield of grain, and higher protein content of the grain. The increased total and available nitrogen in the soil of the spot does not appear to be associated with a more active nitrogen-fixing or nitrifying microflora. Spots identical in appearance and in quantitative measurements have been produced experimentally by the application of cow urine and certain other nitrogen-containing materials. All evidence thus far obtained points to the conclusion that the spots are the direct result of the presence of a more abundant supply of available nitrogen in the soil of these limited areas, particularly during the early spring growing period; and that this increased available nitrogen has arisen from a limited quantity of nitrogen, either already available or capable of being readily transformed into an available condition, finding its way into the soil, in most instances through the deposition of urine.

⁶ GAINES, P. L., SEWELL, M. C., and LATSHAW, W. L. THE NITROGEN BALANCE IN CULTIVATED SEMI-ARID WESTERN KANSAS SOILS. *Jour. Amer. Soc. Agron.* 21: 1130-1153. 1929.

NITROGEN CHANGES PRODUCED IN CERTAIN NITROGENOUS COMPOUNDS BY AZOTOBACTER AND THE NITROGEN FIXED IN THE PRESENCE OF THESE COMPOUNDS¹

By L. G. THOMPSON, Jr.

Formerly Research Fellow, Farm Crops and Soils, Iowa Agricultural Experiment Station²

INTRODUCTION

Soils contain varying amounts of organic nitrogen, mostly in the form of complex organic compounds, but the amount of mineral nitrogen present is usually small. It is therefore believed that most soils do not contain sufficient nitrogen, either organic or mineral, to depress nitrogen fixation by Azotobacter to any large extent. As some of these complex nitrogenous compounds are stimulative to fixation by Azotobacter, a soil rich in organic matter may be more stimulative to nitrogen fixation than a poor soil.

To determine the effect of various nitrogenous compounds on nitrogen fixation, most investigators have measured the amount of nitrogen fixed in the presence of these compounds, but there has been comparatively little study of the nitrogen changes produced, or the amounts of these compounds utilized. In most of this work it has been found that the simple nitrogenous compounds have a greater depressing effect on nitrogen fixation by Azotobacter than the more complex amino acids and proteins. This seems to indicate that the simple nitrogenous compounds may be utilized to a much greater extent than the more complex substances, and hence, in the latter case, the organisms must use the atmospheric nitrogen in order to make a vigorous growth. There is also the possibility that the more complex compounds are more stimulative to growth and hence bring about a greater fixation.

The purpose of the work reported here was to determine the utilization and the nitrogen changes produced by Azotobacter in various nitrogenous compounds, and the effect of these compounds upon the growth and nitrogen fixation of several species of Azotobacter.

REVIEW OF LITERATURE

Numerous investigations have been carried out to determine the effect of nitrogen compounds on the growth of Azotobacter and the fixation of nitrogen.

Hills (10)³ found that the nitrates of potassium, sodium, and calcium increased the growth of Azotobacter, and also the fixation of nitrogen when the amount added was not more than 100 mg per 100 c c of culture medium.

¹ Received for publication Jan. 19, 1932; issued August, 1932. Journal Paper No. B 34 of the Iowa Agricultural Experiment Station. Part of a thesis submitted to the graduate faculty of Iowa State College in partial fulfillment of the requirements for the degree of doctor of philosophy.

² The writer wishes to thank Dr. P. E. Brown for his helpful suggestions in planning the experimental work and in the preparation of the manuscript, and Dr. F. B. Smith for the help which he has given during the progress of the work.

³ Reference is made by number (italic) to Literature Cited, p. 160.

Kostytschew, Ryskaltshuk, and Schwesowa (12) found that the addition of 15 mg of NH_3 nitrogen, 24 mg of NaNO_3 nitrogen, or 130 mg of peptone nitrogen to 100 c c of culture medium practically inhibited nitrogen fixation by *Azotobacter agilis*. They also found small amounts of ammonia in young cultures of this organism and concluded that this was probably the first product of nitrogen fixation.

Lipman (13) noted that 50 mg of potassium nitrate, or 100 mg of peptone per 100 c c of solution depressed nitrogen fixation by *Azotobacter vinelandii*.

Reed and Williams (16) studied the effect of various organic compounds, including many which are toxic to higher plants, on the growth of *Azotobacter* and found that nitrogen fixation was only slightly influenced by most of the compounds used. In concentrations that were fatal to certain higher plants, many of the compounds depressed fixation only slightly. Certain of the compounds, especially urea, glycocoll, formamide, and allantoin at concentrations of 500 parts per million were particularly active in depressing nitrogen fixation. It was suggested that this was not due to toxic effects but rather to the fact that the compounds were utilized by *Azotobacter*.

Winters (19) observed that rapid nitrogen fixation in the soil was at first accompanied by a decrease in nitrate content, which was probably due to the assimilation of nitrates by the organisms. Applications of 100 pounds of sodium nitrate per acre or an equivalent amount of nitrogen as ammonium sulphate or calcium nitrate stimulated nitrogen fixation.

Zoond (20) found that increasing concentrations over 3 mg of nitrate, amino acid, or peptone decreased the amount of nitrogen fixed by *Azotobacter*. Sterile, unheated plant extracts increased nitrogen fixation when applied in moderate amounts.

Lohnis and Green (14) found that manure, straw, and peat stimulated nitrogen fixation when used in a synthetic culture medium.

Murray (15) found that 1 per cent of plant residues when added to soil, greatly stimulated nitrogen fixation. When the same plant materials were added to sterile sand inoculated with *Azotobacter* only a slight stimulation was observed.

Brown and Allison (2), after studying the effect of manure and various plant residues, found that the nitrogen-carbon ratios of the materials used were of little or no value in indicating their effects on nitrogen fixation.

Greaves (7) found that soils comparatively rich in combined nitrogen fixed more nitrogen than did those poorer in organic nitrogen.

Burk and Lineweaver (3) found that the quantity of readily available fixed nitrogen required to inhibit nitrogen fixation by *Azotobacter* was 0.5 mg per 100 c c of solution.

Fuller and Rettger (6) tested the effects of a large number of nitrogen compounds on nitrogen fixation by *Azotobacter* and noted that most of the nontoxic compounds did not influence nitrogen fixation to any great extent. Certain of the compounds such as aspartic acid, cysteine hydrochloride, glycocoll, creatine, creatinine, and urea practically inhibited nitrogen fixation.

The action of *Azotobacter* upon nitrogen-containing compounds has been studied to some extent.

Beijerinck and Van Delden (1) found that *Azotobacter* reduced nitrites directly to ammonia.

Stoklasa et al. (17) found that nitrates were reduced to nitrites and then to ammonia, which was then utilized probably for the building of amino acids and proteins. These findings were confirmed by Kostytschew, Ryskaltshuk, and Schwesowa (12).

Waynick and Woodhouse, as reported by Hunt (11), observed that appreciable amounts of amino nitrogen were present in young cultures of *Azotobacter*.

Halversen (8) found that the amounts of amino nitrogen increased with the age of the culture up to a certain point and then decreased. Nitrites and nitrates could not be detected at any time in vigorously growing cultures, provided the reagents used in making up the solutions were free from these compounds. As ammonia was always found in cultures during the first few days of growth, it was concluded that ammonia was probably the first product of nitrogen fixation.

Fuller and Rettger (6) found that peptone, nucleic acid, aspartic acid, and glyocoll were readily utilized by *Azotobacter*. Tryptophane and glutamic acid were partly utilized, while tryosine was only slightly utilized.

METHODS

The medium used in these studies was nitrogen-free mannitol solution prepared according to the directions of Fred and Waksman (5). The composition of the medium was as follows:

Mannitol.....	10 g	Manganese sulphate.....	Trace
Di-potassium phosphate.....	0.5 g	Ferric chloride.....	Trace
Magnesium sulphate.....	.2 g	Distilled water.....	1,000 c c
Sodium chloride.....	.2 g		

The nitrogenous substance to be tested was added at the rate of 1 g per liter of this medium. The reaction of the medium was adjusted to pH 7.4 by adding a 1 per cent solution of sodium hydroxide, and using brom thymol blue as the indicator. One hundred c c of this medium was added to each of ten 500 c c Erlenmeyer flasks, which were then sterilized and inoculated in duplicate with each of the following organisms: *Azotobacter chroococcum*, *A. beijerinckii*, *A. vinelandii*¹ and *Azotobacter* No. 2. The flasks were incubated at 26° to 28° C. for three weeks, then transfers were made to peptone mannitol agar slants to test the purity of the cultures. Any common contaminating organisms made a very good growth on this medium and could be detected readily. The various forms of nitrogen present in the cultures were determined as quickly as possible at the end of the incubation period.

Alpha amino nitrogen was determined on 6 to 10 c c aliquots with the Van Slyke macro apparatus (18). To increase the accuracy of the apparatus, the macro-gas burette was replaced by a micro-gas burette.

Ammonia nitrogen was determined on 25 c c aliquots by the aeration method. One gram of anhydrous sodium carbonate was added to 25 c c of the culture solution in aeration tubes, which were attached to similar tubes containing a known quantity of standard acid, and the aeration was continued for five hours. The acid was then titrated with standard alkali.

¹ These species were secured through the courtesy of N. R. Smith, Bureau of Chemistry and Soils, U. S. Department of Agriculture.

Nitrites were determined by the alpha-naphthylamine sulphanilic acid method according to Fred and Waksman (5).

Nitrates were determined by the phenoldisulphonic acid method as modified by Harper (9).

Total nitrogen was determined on 25 c c aliquots by the Kjeldahl method. Copper sulphate was used as a catalyst in digestion instead of mercury. When nitrates or nitrites were present in the solutions, they were reduced to ammonia according to the method of Davisson and Parsons (4). Then the digestion and the distillation were carried out in the usual way.

Unless it is stated otherwise, all solutions were incubated for three weeks. The difference in the amount of amino nitrogen in the uninoculated and in the inoculated solutions is given in the tables as the amount of amino nitrogen utilized.

RESULTS

The results in Table 1 show the nitrogen changes produced after three weeks incubation in a solution containing 1 g of KNO_3 per liter. These results show that a large part of the nitrate nitrogen was utilized by the organisms. Only *Azotobacter vinelandii* and *Azotobacter* No. 2 fixed any measurable amount of nitrogen in the presence of this quantity of nitrate. There was a small amount of amino acid nitrogen present in the solutions, indicating that the nitrate probably passed through this form as it was utilized by the organisms.

TABLE 1.—Nitrogen changes produced by various organisms after three weeks incubation in mannitol media containing different chemicals

[Results are expressed as milligrams of nitrogen per 100 c c of solution for the averages of duplicate flasks]

MEDIUM CONTAINING 1 G OF POTASSIUM NITRATE PER LITER

Organism	Amino nitrogen	Ammonia	Nitrites	Nitrates	Total nitrogen
Check	(*)	(*)	(*)	13.7	13.8
<i>Azotobacter chroococcum</i>	1.35	(*)	(*)	3.86	13.8
<i>A. beijerinckii</i>	1.42	(*)	(*)	6.17	13.8
<i>A. vinelandii</i>	2.10	(*)	(*)	3.03	15.5
<i>Azotobacter</i> No. 2	2.10	(*)	(*)	(*)	14.6

MEDIUM CONTAINING 1 G OF AMMONIUM SULPHATE PER LITER

Check	3.8	21.4	(*)	(*)	21.2
<i>Azotobacter chroococcum</i>	4.03	18.5	(*)	(*)	20.8
<i>A. beijerinckii</i>	4.03	19.1	(*)	(*)	20.4
<i>A. vinelandii</i>	4.74	18.8	(*)	(*)	22.6
<i>Azotobacter</i> No. 2	3.9	19.1	(*)	(*)	20.4

MEDIUM CONTAINING 1 G OF UREA PER LITER

Check		3.38	(*)	(*)	47.2
<i>Azotobacter chroococcum</i>		17.6	(*)	(*)	38.8
<i>A. beijerinckii</i>		12.4	(*)	(*)	39.6
<i>A. vinelandii</i>		24.4	(*)	(*)	34.8
<i>Azotobacter</i> No. 2		10.1	(*)	(*)	48.4

* None.

† Trace.

As there was a trace of nitrite in the culture of *Azotobacter beijerinckii*, it was decided to test for nitrites and ammonia after short periods of incubation. Sodium nitrate was used in this experiment as it seemed to give somewhat better growth and might therefore permit of the accumulation of nitrites and ammonia.

TABLE 2.—*Nitrogen changes produced by various organisms in a mannitol medium containing 1 g of NaNO₃ per liter after various periods of incubation*

[Results expressed as milligrams of nitrogen per 100 c c of solution for averages of duplicate flasks]

Organism	Nitrogen in form indicated produced after incubation of—								
	4 days			6 days			8 days		
	Ni- trates	Ni- trites	Ammo- nia	Ni- trates	Ni- trites	Ammo- nia	Ni- trates	Ni- trites	Ammo- nia
Check.....	13.7	(a)	(a)	13.8	(a)	(a)	13.6	(a)	(a)
<i>Azotobacter chroococcum</i>	12.3	(b)	(b)	8.1	(b)	(b)	6.2	(a)	(b)
<i>A. beijerinckii</i>	12.9	0.02	0.28	9.9	0.02	0.28	7.8	(b)	0.14
<i>A. vinelandii</i>	12.4	(b)	.56	8.2	.36	1.4	6.4	(a)	.14
<i>Azotobacter</i> No. 2.....	10.4	(b)	(b)	7.1	.71	.56	2.3	0.02	.42
									14.10

The results in Table 2 show the changes produced in a solution containing 1 g of NaNO₃ per liter. There was a measurable accumulation of nitrites and ammonia in the cultures, which indicates that the nitrates were reduced to nitrites and then to ammonia. Probably the reason there was not a larger accumulation of nitrites and ammonia was because of a rapid utilization. The total nitrogen determinations at the end of 14 days show no loss of nitrogen indicating that all the nitrates were utilized by the bacteria, or, if there was a loss, the difference was made up by nitrogen fixation. It would seem more reasonable to believe that the nitrates were used in preference to free nitrogen and that there was no fixation of free nitrogen during the reduction process. In this experiment *Azotobacter* No. 2 did not utilize all the nitrate nitrogen. In some other experiments where the growth was somewhat better, all the nitrate was utilized in about seven to eight days in the case of this organism.

The different species of *Azotobacter* were grown in methylene blue whole milk, and it was found that the organisms which reduced nitrates to the greatest extent also reduced methylene blue in a shorter period of time.

A test was also made to determine whether they would grow on nitrate agar in sealed tubes. No growth occurred, while there was excellent growth on the check tubes, which were not sealed. Probably these organisms reduce nitrates in order to secure the nitrogen and not the oxygen.

Nitrites added to a mannitol solution at the rate of 10 mg per liter were practically all reduced in two days, and after three days they were all reduced by these four species of *Azotobacter*.

Table 1 shows the nitrogen changes produced in a solution containing 1 g of ammonium sulphate per liter. There was no fixation of nitrogen except possibly in the case of *Azotobacter vinelandii*, but there

was a slight loss of nitrogen probably as ammonia. While the figures for amino nitrogen include considerable ammonia nitrogen, they are at least comparable. The Van Slyke method gives results in the presence of ammoniacal nitrogen which are comparable if the time of making all the determinations is the same. The ammonia reacts slowly so that if three minutes are used for making the determinations, about 20 per cent of the ammonia is included in the amino nitrogen determinations. As all the determinations reported here were made in three minutes, the results are at least comparable. All the cultures contained less ammonia nitrogen than did the check, but more amino nitrogen, which indicates that there was a measurable amount of amino nitrogen present, especially in the cultures of *A. vinelandii*.

As no nitrates or nitrites could be detected, the ammonia must have been used by the organisms. The growth was not very good, which indicates that the sulphuric acid remaining after the utilization of the ammonia, made the medium more acid than was favorable for the growth of the organisms.

In the solution containing 1 g of urea per liter, a large amount of ammonia was produced which shows that all the organisms were very active in decomposing urea. Tests of these cultures would probably show the presence of a large quantity of the enzyme urease, which is active in changing urea to ammonia. *Azotobacter vinelandii* was especially active in changing urea to ammonia, while *A. chroococcum* was almost as active. There was a loss of nitrogen from all the cultures except *Azotobacter* No. 2, and in this culture there was a slight fixation. The quantity of nitrogen fixed was too small, however, to be significant. There was less ammonia produced in this culture than in any of the other cultures, so there probably was no loss of ammonia, or, if there was a loss, it was made up by nitrogen fixation. There is a correlation between the amount of nitrogen lost and the amount of ammonia produced, e. g., the cultures containing the most ammonia contained the least total nitrogen. This shows that the nitrogen was undoubtedly lost in the form of ammonia.

In the Van Slyke method the urea as well as the ammonia reacts slowly with the nitrous acid to give free nitrogen gas, so that the results for amino nitrogen include a part of the ammonia and also a part of the urea nitrogen. For this reason the figures are not given here. It may be stated, however, that the quantity of amino nitrogen, as determined under these conditions, was very much higher in the inoculated than in the uninoculated cultures, which indicates that there may have been considerable amino nitrogen present.

Table 3 shows the nitrogen changes produced in mannitol medium containing 1 g of α -alanine per liter. The only organism that used alanine to any extent was *Azotobacter vinelandii*. *Azotobacter* No. 2 used a very small amount, but this amount is probably not significant. In the three instances where alanine was not utilized to any extent, it seemed to stimulate nitrogen fixation slightly. Where alanine was utilized, however, there was a depressing effect on nitrogen fixation. The amount of nitrogen fixed, both in the presence and in the absence of alanine, was rather low, probably because there was no calcium in the medium. Tests made after this experiment was completed show that all these organisms will fix considerably more nitrogen in the presence of calcium. There was a small amount of ammonia produced by *A. vinelandii* but this may have been within the

experimental variation. It may also be possible that the alanine was decomposed to ammonia before it was utilized by the organism.

TABLE 3.—Nitrogen changes produced by various organisms in a mannitol media containing various amino acids and nitrogen compounds

[Results are expressed as milligrams of nitrogen per 100 c.c. of solution for the averages of duplicate flasks]

MEDIUM CONTAINING 1 G OF α -ALANINE PER LITER

Organism	Nitrogen fixed	Amino nitrogen	Amino nitrogen utilized	Ammonia	Nitrogen-free medium nitrogen fixed	Total nitrogen
Check	—	15.9	—	(*)	—	16.0
<i>Azotobacter chroococcum</i>	2.6	15.9	0	(*)	—	16.6
<i>A. beijerinckii</i>	1.8	16.4	— .5	(*)	1.8	17.8
<i>A. vinelandii</i>	4.0	13.3	2.6	1.56	1.6	20.0
<i>Azotobacter</i> No. 2	5.4	15.5	.4	(*)	3.4	21.4

MEDIUM CONTAINING 1 G OF DL-VALINE PER LITER

Organism	Nitrogen fixed	Amino nitrogen	Amino nitrogen utilized	Ammonia	Nitrogen-free medium nitrogen fixed	Total nitrogen
Check	—	15.4	—	(*)	—	15.6
<i>Azotobacter chroococcum</i>	0.6	14.2	1.2	(*)	1.8	16.2
<i>A. beijerinckii</i>	4.3	14.7	.7	(*)	1.6	19.9
<i>A. vinelandii</i>	.1	13.6	1.8	(*)	5.7	15.7
<i>Azotobacter</i> No. 2	.9	13.8	1.6	(*)	3.4	16.5

MEDIUM CONTAINING 1 G OF DL- α -AMINO-N-BUTYRIC ACID PER LITER

Organism	Nitrogen fixed	Amino nitrogen	Amino nitrogen utilized	Ammonia	Nitrogen-free medium nitrogen fixed	Total nitrogen
Check	—	14.5	—	(*)	—	14.6
<i>Azotobacter chroococcum</i>	0.6	14.8	— 0.3	(*)	1.8	15.2
<i>A. beijerinckii</i>	3.9	14.8	— .3	(*)	1.6	18.5
<i>A. vinelandii</i>	.9	12.3	2.2	0.56	5.7	15.5
<i>Azotobacter</i> No. 2	2.2	14.5	0	(*)	3.4	17.8

MEDIUM CONTAINING 1 G OF ASPARAGIN PER LITER

Organism	Nitrogen fixed	Amino nitrogen	Amino nitrogen utilized	Ammonia	Nitrogen-free medium nitrogen fixed	Total nitrogen
Check	—	11.1	—	(*)	—	20.5
<i>Azotobacter chroococcum</i>	1.3	11.5	— 0.4	5.32	1.8	21.8
<i>A. beijerinckii</i>	.4	11.6	— .5	5.04	1.6	20.9
<i>A. vinelandii</i>	.9	7.7	3.4	6.16	5.7	21.4
<i>Azotobacter</i> No. 2	.7	10.8	.3	3.92	3.4	21.2

MEDIUM CONTAINING 2 G OF EGG ALBUMIN PER LITER

Organism	Nitrogen fixed	Amino nitrogen	Amino nitrogen utilized	Ammonia	Nitrogen-free medium nitrogen fixed	Total nitrogen
Check	—	(*)	(*)	(*)	—	24.8
<i>Azotobacter chroococcum</i>	3.8	(*)	(*)	(*)	1.8	28.6
<i>A. beijerinckii</i>	3.8	(*)	(*)	(*)	1.6	28.6
<i>A. vinelandii</i>	3.0	(*)	(*)	(*)	5.7	27.8
<i>Azotobacter</i> No. 2	5.3	(*)	(*)	(*)	3.4	30.1

MEDIUM CONTAINING 2 G OF PEPTONE PER LITER

Organism	Nitrogen fixed	Amino nitrogen	Amino nitrogen utilized	Ammonia	Nitrogen-free medium nitrogen fixed	Total nitrogen
Check	—	8.17	—	(*)	—	34.8
<i>Azotobacter chroococcum</i>	2.7	7.33	0.84	(*)	1.8	37.5
<i>A. beijerinckii</i>	2.9	8.09	.08	(*)	1.4	37.7
<i>A. vinelandii</i>	3.0	5.57	2.60	0.56	5.7	37.8
<i>Azotobacter</i> No. 2	2.6	7.49	.08	(*)	3.4	37.4

MEDIUM CONTAINING 2 G OF CASEIN PER LITER

Organism	Nitrogen fixed	Amino nitrogen	Amino nitrogen utilized	Ammonia	Nitrogen-free medium nitrogen fixed	Total nitrogen
Check	—	3.65	—	(*)	—	34.5
<i>Azotobacter chroococcum</i>	1.6	3.49	0.16	(*)	1.8	36.1
<i>A. beijerinckii</i>	.9	2.72	.93	(*)	1.6	35.6
<i>A. vinelandii</i>	2.5	2.31	1.34	(*)	5.7	37.0
<i>Azotobacter</i> No. 2	2.7	3.68	— .03	(*)	3.4	37.2

* None.

TABLE 3.—Nitrogen changes produced by various organisms in a mannitol media containing various amino acids and nitrogen compounds—Continued

MEDIUM CONTAINING 1 G OF L-TYROSINE PER LITER

Organism	Nitrogen fixed	Amino nitrogen	Amino nitrogen utilized	Ammonia	Nitrogen-free medium nitrogen fixed	Total nitrogen
Check		9.1		(a)		8.9
<i>Azotobacter chroococcum</i>	2.2	9.3	-0.2	(a)	2.5	11.1
<i>A. beijerinckii</i>	1.9	9.2	-1	(a)	2.2	10.8
<i>A. vinelandii</i>	10.1	7.9	1.2	(a)	8.6	19.0
<i>Azotobacter</i> No. 2	3.1	9.0	.1	(a)	5.9	12.0

MEDIUM CONTAINING 1 G OF D-GLUTAMIC ACID PER LITER

Check		9.80		(a)		9.69
<i>Azotobacter chroococcum</i>	5.20	9.52	0.28	(a)	2.5	14.89
<i>A. beijerinckii</i>	.56	9.91	-11	(a)	2.2	10.25
<i>A. vinelandii</i>	2.80	5.94	3.86	(a)	8.6	12.49
<i>Azotobacter</i> No. 2	(a)	6.32	3.48	(a)	5.9	9.69

MEDIUM CONTAINING 1 G OF DL-A-AMINO-N-VALERIC ACID PER LITER

Check		12.6		(a)		12.40
<i>Azotobacter chroococcum</i>	2.07	12.7	-0.1	(a)	2.5	14.47
<i>A. beijerinckii</i>	1.68	12.9	-3	(a)	2.2	14.08
<i>A. vinelandii</i>	3.38	10.5	2.1	(a)	8.6	15.76
<i>Azotobacter</i> No. 2	3.20	11.2	1.4	(a)	5.9	15.60

MEDIUM CONTAINING 1 G OF DL-PHENYLALANINE PER LITER

Check		8.70		(a)		8.64
<i>Azotobacter chroococcum</i>	3.08	7.80	0.90	(a)	2.5	11.72
<i>A. beijerinckii</i>	2.10	8.60	.10	(a)	2.2	10.74
<i>A. vinelandii</i>	8.70	7.70	1.00	(a)	8.6	17.34
<i>Azotobacter</i> No. 2	5.74	7.75	.95	(a)	5.9	14.38

(a) None.

Glycocoll at a concentration of 1 g per liter was toxic to the organisms, and very poor growth was secured. The amounts of amino nitrogen utilized or the amounts of nitrogen fixed were very small, so the figures are not given here. At lower concentrations glycocoll probably would not be toxic and might be utilized by the organisms.

The results in Table 3 show that where the utilization of dl-valine was greatest, the amount of nitrogen fixed was the smallest. The very small amount of the amino nitrogen used by *Azotobacter beijerinckii* was probably not significant. This seems to indicate that *A. beijerinckii* could not use the valine and hence had to secure its nitrogen from the atmosphere. The valine seemed to be very stimulative to its growth. In the case of the other organisms, however, the valine depressed nitrogen fixation, probably not because it was toxic, but because it was utilized in preference to free nitrogen.

A mannitol medium containing 1 g of dl-a-amino-n-butyric acid per liter depressed nitrogen fixation by all the organisms except *Azotobacter beijerinckii* in which it stimulated fixation. This seems to indicate that it must have been toxic at this concentration to all the organisms except *A. beijerinckii*. In the case of *A. vinelandii*, however, the

depressed fixation may have been due to the utilization of the amino acid in preference to free nitrogen. It seems that *A. beijerinckii* can withstand a higher concentration of this amino acid without a toxic effect than is true of the other organisms. Many compounds that are toxic at higher concentrations are stimulative at lower concentrations. The results seem to indicate that this may be the case with many of the amino acids, especially if they are not utilized.

The results show that asparagin was utilized only by *Azotobacter vinelandii* and *Azotobacter* No. 2. In the inoculated solutions, the quantities of amino nitrogen were higher than they should be because part of the ammonia nitrogen was included in the determinations. As 6 c c aliquots were used in making the amino acid determinations, the quantity of ammonia present would be less than 1 mg, and 21 per cent of this amount would be about 0.2 mg (the percentage of ammonia nitrogen when the time of making the determinations is three minutes). As each of these determinations was made in three minutes, the amount of ammonia included would be small, e. g., not more than 0.3 or 0.4 mg. The extra amount of amino nitrogen present in the cultures of *A. chroococcum* and *A. beijerinckii* over that of the check was about equal to the amount of ammonia included in the determinations. It is, therefore, believed that there was no utilization of amino nitrogen in the case of these two organisms.

The amide nitrogen was readily attacked by all the organisms, with the production of large amounts of ammonia, which was then utilized by the organisms in preference to free nitrogen. This indicates that the amide nitrogen was very effective in depressing fixation. Tests were not made with asparic acid, but in all probability all the organisms except *Azotobacter vinelandii* would have fixed considerable amounts of nitrogen.

The egg albumin stimulated nitrogen fixation by all the organisms except *Azotobacter vinelandii* in which it depressed fixation. This seems to indicate that the egg albumin could not be utilized by any of the organisms except *A. vinelandii*. This organism may have used the protein in preference to fixing nitrogen, or the protein may have been slightly toxic. As *A. vinelandii* used practically every amino acid tested, there is good reason to believe that it used the egg albumin also. No amino acid could be detected in any of the cultures when the Van Slyke method was used to make the determinations. This indicates that the organisms were unable to break down the egg albumin into amino acids, or, if they did, the amino acids were all utilized by the end of the three weeks' period. Ammonia was not detected in any of the cultures.

The peptone stimulated fixation by all the organisms except *Azotobacter vinelandii* and *Azotobacter* No. 2. The depressed fixation in these cases was probably due to the fact that these organisms used the amino acids in preference to the free nitrogen. *A. chroococcum* and *Azotobacter* No. 2 utilized a small amount of amino nitrogen, but *A. beijerinckii* used practically none. The presence of the peptone, however, seemed to stimulate this organism to fix much more nitrogen. There was a small amount of ammonia produced by *A. vinelandii*, but this amount may not be significant. There was also the possibility that the amino acids were decomposed to ammonia and then utilized by *A. vinelandii*.

In a mannitol medium containing 2 g of casein per liter, nitrogen fixation was depressed in all cases, but it was depressed to a greater extent in the case of *Azotobacter vinelandii* and *A. beijerinckii*. This was probably due to the fact that these two organisms used the amino nitrogen instead of free nitrogen. *A. chroococcum* and *Azotobacter* No. 2 utilized practically none of the amino nitrogen, and fixation was only very slightly depressed.

Because of the low nitrogen fixation secured in the previous work, it was decided to use calcium carbonate in the following experiments. The same medium was used, except that 3 g of CaCO_3 per liter was added.

L-tyrosine was not utilized by any of the organisms except *Azotobacter vinelandii*, but it seemed to have a depressing effect on nitrogen fixation by all the organisms except *A. vinelandii*, in which there was a slight stimulation. Although the amino acid was utilized by *A. vinelandii*, it did not depress fixation. This may be due to the phenol group, which seemed to stimulate this organism to a greater growth but was slightly toxic to the other organisms. Probably at a higher concentration this substance would have been toxic to *A. vinelandii*, whereas at a lower concentration it may have stimulated the other organisms.

All the organisms utilized d-glutamic acid, except *Azotobacter beijerinckii*, but the amount used by *A. chroococcum* was very small. *A. chroococcum* fixed a large amount of nitrogen, which shows that the amino acid was stimulative to this organism. The amino acid was undoubtedly toxic to *A. beijerinckii*, however, as it made a very poor growth. In the case of *A. vinelandii* and *Azotobacter* No. 2, nitrogen fixation was greatly depressed, probably because the amino acid was utilized in preference to free nitrogen.

A mannitol medium containing 1 g of dl- α -amino-n-valeric acid per liter depressed nitrogen fixation only slightly in the case of *Azotobacter chroococcum* and *A. beijerinckii*, but it was very effective in depressing nitrogen fixation in the other two organisms. This was probably due to the fact that *A. chroococcum* and *A. beijerinckii* were unable to use the amino nitrogen and hence had to depend on the process of fixation to secure their nitrogen, whereas the other two organisms used the amino nitrogen as well as the free nitrogen.

The amounts of dl-phenylalanine utilized were very small, except in the case of *Azotobacter vinelandii*, in which there was considerable utilization. The amounts used in the cultures of *A. beijerinckii* were not significant. The phenylalanine did not depress nitrogen fixation, and in the case of *A. chroococcum* there was a slight stimulation. Ammonia was not produced by any of the organisms. The results seem to show that the amino acid was very stimulative to the growth of all the organisms except *A. beijerinckii*, and that in making a vigorous growth the organisms used a large amount of free nitrogen, for they were unable to use any considerable amount of the fixed nitrogen.

Table 4 shows the amounts of nitrogen fixed in a nitrogen-free mannitol medium with and without 1 g of l-cystine per liter. The amounts of nitrogen fixed were slightly higher in all the cultures containing l-cystine than in the nitrogen-free medium. Determinations of amino nitrogen were not made, but it is thought that there was little or no utilization of the cystine for it was insoluble in the medium.

TABLE 4.—*Nitrogen fixed by various organisms in a nitrogen-free mannitol medium and in the same medium containing 1 g of l-cystine per liter*

[Results expressed as milligrams of nitrogen per 100 c c of medium for the averages of duplicate flasks]

Organism	Medium +cystine	Medium alone
<i>Azotobacter chroococcum</i>	2.06	2.5
<i>A. beijerinckii</i>	2.52	2.2
<i>A. vinelandii</i>	8.96	8.6
<i>Azotobacter</i> No. 2.....	6.00	5.9

DISCUSSION

The results as a whole show that the simple nitrogenous compounds have a greater depressing effect on nitrogen fixation than do the more complex ones due to the fact that the simple compounds are readily utilized by the organisms, whereas many of the complex compounds are only slightly utilized, or in most cases not utilized at all. When the complex compounds depressed fixation, it was generally found that they were either utilized by the organisms or that they were toxic to growth. The amino acids contained in peptone were not utilized to any large extent, except by *Azotobacter vinelandii*, but they stimulated nitrogen fixation in some cases. Certain of the other compounds brought about a stimulation in the fixation by some of the organisms. D-glutamic acid was found to stimulate *A. chroococcum* to a large extent, but it depressed the fixation by all the other organisms. Practically all the compounds tested were utilized to some extent by *A. vinelandii*, and as a result nitrogen fixation was depressed to a greater or less extent. Although l-tyrosine and dl-phenylalanine were found to be utilized by *A. vinelandii*, nitrogen fixation was not depressed. As these were the only instances in which the compounds were utilized and at the same time did not depress nitrogen fixation, it is doubtful whether they were utilized in preference to the free nitrogen. The organism used only about one-eighth of the amino nitrogen present but at the same time fixed from 9 to 10 mg of nitrogen. This seems to show that these two compounds were very stimulative to growth, and that the organism, being unable to use any considerable amount of the amino nitrogen, utilized the free nitrogen for its growth.

Certain of the amino acids, such as d-glutamic acid, dl-a-amino-n-valeric acid, and dl-valine, were utilized in part by *Azotobacter* No. 2. *Azotobacter chroococcum* was able to utilize some of the amino nitrogen in the compounds such as dl-phenylalanine, dl-valine, and peptone, while *A. beijerinckii* used some of the amino nitrogen of valine and casein. Of the amino acids tested, however, the number as well as the amount utilized by the organisms except *A. vinelandii* was exceedingly small. *A. vinelandii* used practically all of the amino acids tested, but the amounts used were rather small.

The nitrates, nitrites, urea, and the amide nitrogen of asparagin were readily attacked with the production of ammonia which was then utilized by the organisms. A small amount of ammonia was found in the cultures containing certain amino acids, which indicates that certain of the amino acids may be decomposed to ammonia before they are utilized by the organisms. Other amino acids may be of such a structure that they can be utilized directly.

With very few exceptions, all the compounds that were readily utilized inhibited nitrogen fixation entirely. Compounds that were utilized with difficulty depressed nitrogen fixation usually to the extent that they were utilized. In a few cases these latter compounds depressed fixation to a greater extent, and in others they stimulated fixation to a greater or less degree. When the compounds were not utilized, they had little or no effect or they were stimulative to fixation. In a few instances the compounds were toxic at the concentration used and hence greatly depressed growth and nitrogen fixation.

Nitrates usually stimulated pigment formation, but it was possible to secure a good growth in a nitrate solution without pigment formation. In fact, growth seemed to be better in cultures where there was no pigment formation. After pigment was formed, even in a nitrate solution, the growth seemed to be slower, indicating some unfavorable conditions.

SUMMARY

The nitrogen changes produced by four species of *Azotobacter* in mannitol media containing some 17 different nitrogenous compounds were studied. The amounts of these compounds utilized by the organisms as well as their effect on nitrogen fixation were determined.

The nitrates of sodium and potassium, potassium nitrite, urea, and the amide nitrogen of asparagin were readily attacked with the production of ammonia, which was then utilized by the organisms. These compounds inhibited nitrogen fixation almost entirely, and in some cases there was a loss of ammonia.

All the organisms tested, except *Azotobacter vinelandii*, utilized only a few of the amino acids, and where there was utilization the amount used was very small. *A. vinelandii* utilized practically every amino acid tested, but the amount used was not very large. Where the amino acids were utilized, nitrogen fixation was usually depressed. If the amino acids were not utilized, nitrogen fixation was generally either stimulated or affected not at all. The simple nitrogenous compounds were more readily utilized than the complex compounds.

With the exception of *Azotobacter vinelandii* the organisms that fixed the most nitrogen when grown in a nitrogen-free medium, used the largest amount of nitrate nitrogen when grown in a medium containing nitrates. *A. vinelandii* fixed a large amount of nitrogen when grown on nitrogen-free medium but did not utilize quite all of the nitrates when grown in a medium rich in nitrate nitrogen. In the latter medium it fixed a small amount of nitrogen.

Azotobacter No. 2 was able to utilize 14 mg of nitrate nitrogen per 100 c c of medium in seven to nine days.

Many of the complex compounds were not utilized except to a small extent by *Azotobacter vinelandii*, and nitrogen fixation was usually not affected or was stimulated.

LITERATURE CITED

- (1) BEIJERINCK, M. W., and DELDEN, A. VAN
1902. ÜBER DIE ASSIMILATION DES FREIEN STICKSTOFFS DURCH BAKTERIEN.
Centbl. Bakt. [etc.] (II) 9:3-43.
- (2) BROWN, P. E., and ALLISON, F. E.
1916. THE INFLUENCE OF SOME HUMUS-FORMING MATERIALS OF NARROW
AND OF WIDE NITROGEN-CARBON RATIO ON BACTERIAL ACTIVITIES. Soil Sci. 1:49-75.

- (3) BURK, D., and LINEWEAVER, H.
1930. THE INFLUENCE OF FIXED NITROGEN ON AZOTOBACTER. *Jour. Bact.* 19:389-414, illus.
- (4) DAVISSON, B. S., and PARSONS, J. T.
1919. THE DETERMINATION OF TOTAL NITROGEN INCLUDING NITRIC NITROGEN. *Jour. Indus. and Engin. Chem.* 11:306-311, illus.
- (5) FRED, E. B., and WAKSMAN, S. A.
1928. LABORATORY MANUAL OF GENERAL MICROBIOLOGY, WITH SPECIAL REFERENCE TO THE MICROORGANISMS OF THE SOIL. 145 p., illus. New York.
- (6) FULLER J. E., and RETTGER, L. F.
1931. THE INFLUENCE OF COMBINED NITROGEN ON GROWTH AND NITROGEN FIXATION BY AZOTOBACTER. *Soil Sci.* 31:219-234.
- (7) GREAVES, J. E.
1914. A STUDY OF THE BACTERIAL ACTIVITIES OF VIRGIN AND CULTIVATED SOILS. *Centbl. Bakt. [etc.] (II)* 41:444-459.
- (8) HALVERSEN, W. V.
1927. THE NITROGEN METABOLISM OF NITROGEN-FIXING BACTERIA. *Iowa State Col. Jour. Sci.* 1:395-410.
- (9) HARPER, H. J.
1924. THE ACCURATE DETERMINATION OF NITRATES IN SOILS PHENOL-DISULFONIC ACID METHOD. *Jour. Indus. and Engin. Chem.* 16:180-183.
- (10) HILLS, T. L.
1918. INFLUENCE OF NITRATES ON NITROGEN-ASSIMILATING BACTERIA. *Jour. Agr. Research* 12:183-230.
- (11) HUNT, T. F.
1919. BY WHAT STEPS DOES AZOTOBACTER FIX NITROGEN? *Calif. Agr. Expt. Sta. Rpt.* 1918-19:62-63.
- (12) KOSTYTSCHEW, S., RYSKALTSCHUK, A., and SCHWEZOWA, O.
1926. BIOCHEMISCHE UNTERSUCHUNGEN ÜBER AZOTOBACTER AGILE. *Hoppe-Seyler's Ztschr. Physiol. Chem.* 154:1-17.
- (13) LIPMAN, J. G.
1905. AZOTOBACTER STUDIES. *N. J. Agr. Expt. Sta. Ann. Rpt.* (1905) 18:254-280.
- (14) LÖHNIS, F., and GREEN, H. H.
1914. ÜBER DIE ENTSTEHUNG UND DIE ZERSETZUNG VON HUMUS, SOWIE ÜBER DESSEN EINWIRKUNG AUF DIE STICKSTOFF-ASSIMILATION. *Centbl. Bakt. [etc.] (II)* 40:52-60.
- (15) MURRAY, T. J.
1917. PART I. THE EFFECT OF DIFFERENT PLANT TISSUES ON THE FIXATION OF ATMOSPHERIC NITROGEN. *Va. Agr. Expt. Sta. Tech. Bul.* 15:93-102.
- (16) REED, H. S., and WILLIAMS, B.
1915. THE EFFECT OF SOME ORGANIC SOIL CONSTITUENTS UPON NITROGEN FIXATION BY AZOTOBACTER. *Va. Agr. Expt. Sta. Tech. Bul.* 4, p. 81-95.
- (17) STOKLASA, J.
1908. BEITRAG ZUR KENNTNIS DER CHEMISCHEN VORGÄNGE BEI DER ASSIMILATION DES ELEMENTAREN STICKSTOFFS DURCH AZOTOBACTER UND RADIOBACTER. *Centbl. Bakt. [etc.] (II)* 21:484-509, 620-632, illus.
- (18) VAN SLYKE, D. D.
1912. THE QUANTITATIVE DETERMINATION OF ALIPHATIC AMINO GROUPS. II. *Jour. Biol. Chem.* 12:275-284, illus.
- (19) WINTERS, N. E.
1924. SOIL CONDITIONS WHICH PROMOTE NITROGEN FIXATION. *Jour. Amer. Soc. Agron.* 16:701-716.
- (20) ZOOND, A.
1926. THE RELATION OF COMBINED NITROGEN TO THE PHYSIOLOGICAL ACTIVITY OF AZOTOBACTER. *Brit. Jour. Expt. Biol.* 4:[105]-113, illus.

THE EFFECT OF THE AMOUNT OF FEED CONSUMED BY CATTLE ON THE UTILIZATION OF ITS ENERGY CONTENT¹

By H. H. MITCHELL, *Chief in Animal Nutrition*, and T. S. HAMILTON, *Associate in Animal Nutrition*, with the technical assistance of F. J. McCLURE, W. T. HAINES, JESSIE R. BEADLES, and H. P. MORRIS, *Illinois Agricultural Experiment Station*

INTRODUCTION

The problem of assessing the value of a feed as a source of energy in nutrition, either in terms of net energy, total digestible nutrients, or other conceptions possessing an absolute rather than a comparative significance, is complicated by the fact that the utilization of the feed energy, either in digestion or in metabolism or both, is conditioned to some extent by the amount of feed consumed. Considerable evidence exists in the literature proving, for rations consisting entirely or to a large extent of concentrates, that digestion is more complete at low levels of intake than at high levels. For this reason, it has frequently been noted in experiments planned so as to allow definite amounts of digestible nutrients to the experimental animals, these amounts to be estimated on the basis of average digestion coefficients, that the digestible nutrients actually consumed, as determined in later digestion trials, are considerably smaller in amount than expected. The reason is that many if not most digestion coefficients have been determined at low levels of feeding.

The amount of food consumed has also been shown to affect the proportion of the food energy lost as animal heat, the so-called "heat increment," or the specific dynamic effect. In order to alleviate to some extent the discomfort of experimental human subjects undergoing basal metabolism measurements, occasioned by abstention from food for 12 to 15 hours, Soderstrom, Barr, and DuBois (20)² and later Benedict and Benedict (3) have studied the effects on heat production of small breakfasts containing 200 to 250 calories. It was found by the former investigators that, after one hour from the time of eating, the effect of the small meal on heat production was inconsiderable; the latter investigators were not able to establish any consistent effect of their meal on two subjects. With one subject, a woman, the metabolism was certainly not raised by the ingestion of food in two tests, even at 15 minutes after eating; in a third test it was raised from 5 to 7 per cent for a period of 40 to 60 minutes. With the second subject, a man, in the only test made, the influence of eating amounted to somewhat more than 10 per cent in the first 15-minute period, but at the time of the next determination, 40 minutes after eating, basal heat production had again established itself.

In two publications appearing in 1928 (9) and 1930 (10), Forbes and his associates reported the results of experiments on four steers, designed to measure the effect on heat production of increasing a

¹ Received for publication Oct. 9, 1931; issued August, 1932.

² Reference is made by number (italic) to Literature Cited, p. 190.

constant ration of corn meal and alfalfa hay, consisting of equal parts of dry matter from each feed, from a half-maintenance to a twice-maintenance or, in one case, to a thrice-maintenance level. They confirmed the results of Soderstrom, Barr, and DuBois and of Benedict and Benedict indicating an inappreciable effect on heat production of the ingestion of small amounts of food. From a level of 1.5 times maintenance upward, the heat production curves were approximately linear, though slight depressions in the curves, of uncertain significance, occurred at different levels of feeding. The investigators themselves describe the curve of heat production in relation to the plane of nutrition as a reversed or S curve. However, the significance of the downward trends at the higher levels of nutrition may be questioned, both because they are in all cases slight and because they occur at different levels of feeding when noticeable at all. In steer No. 47 a slight bend in the curve may be noted at a level of 1.5 times maintenance, but in its mate, steer No. 36, this bend is hardly perceptible. For steer No. 57 a bend in the curve occurs at a level of twice maintenance, but for steer No. 60 it is deferred until a level 2.5 times the maintenance requirements is reached.

Wiegner and Ghoneim (21), using a rabbit for their experiments, have reported an investigation of the utilization of the metabolizable energy of a constant ration at five different levels of intake. Only the highest level of feeding permitted a positive energy balance. The net energy of each level of food was computed as the difference between the energy balance of the period and the energy balance of fasting. The percentage net availability of the metabolizable energy decreased progressively from the lowest to the highest level of feeding, and the relation between intake of metabolizable energy and intake of net energy was a linear one. All data cited are expressed in terms of calories per square meter of body surface, and no reason or defense is given for this unique method of expression.

The relation of heat production to the level of feeding at high levels is of considerable practical importance in animal nutrition, corresponding as these levels do to the most productive feeding. The Pennsylvania experiments throw considerable light upon this relationship, and the experiments to be described below are concerned with the same subject. They were started before the Pennsylvania work was published.

DESCRIPTION OF THE EXPERIMENT

The purpose of the experiment to be described in this report is much the same as that of the Pennsylvania experiments, namely, to determine the effect of the amount of feed consumed upon the utilization of its energy. Four grade Shorthorn steers, approximately 2 years of age, were started on experiment, but since only one of them could be induced to consume enough feed under laboratory conditions to warrant continued study, the other three were soon discarded.

It was planned to select an experimental ration solely on the basis of its palatability. The ration finally selected, in the amounts constituting a full feed as determined by the animal's capacity to consume it, contained 10 kg of corn, 3 kg of alfalfa hay, 250 g of linseed meal, and 116 g of molasses. On the dry-matter basis, this ration contained, as the average of analyses in the five experimental

periods, 73.1 per cent of ground corn, 24.1 per cent of alfalfa hay, 2.0 per cent of linseed meal, and 0.8 per cent of molasses.

The five experimental periods involve observations on the digestibility and energy utilization of this ration at levels of full feed, as defined above, four-fifths feed, three-fifths feed, two-fifths feed, and one-fifth feed. The fasting metabolism of the steer was determined in two additional periods.

The chronological order of the experimental periods, their lengths and pertinent dates, all in the winter and spring months of 1928, are given in Table 1.

TABLE 1.—*Schedule of experiments*

Ration fed (grams)				Time on feed		
Corn	Alfalfa hay	Linseed meal	Molasses	Initial date	Final date	Days
8,000	2,400	200	100	Jan. 19.	Feb. 14.	27
10,000	3,000	250	116	Feb. 17.	Mar. 11.	* 21
6,000	1,800	150	75	Mar. 20.	Apr. 5.	17
0	0	0	0			
4,000	1,200	100	50	Apr. 20.	May 10.	21
2,000	600	50	25	May 11.	May 31.	21
0	0	0	0			

Collection period			Days in respiration chamber			
Initial date	Final date	Days	First dates	Second dates	Third dates	Experiment No.
Jan. 30	Feb. 12	14	Feb. 1-2.	Feb. 3-4.	Feb. 13-14.	7, 8, 9.
Mar. 7	Mar. 11	5	Feb. 28-29	Mar. 1-2.	Mar. 9-10.	10, 11, 12.
Mar. 27	Apr. 5	10	Mar. 30-31	Apr. 2-3.	Apr. 5-6.	13, 14, 15.
			Apr. 16-17.	Apr. 17-18.	Apr. 19-20.	16, 17, 18. ^b
May 1	May 10	10	May 2-3.	May 8-9.	May 9-10.	19, 20, 21.
May 20	May 29	10	May 21-22.	May 22-23.	May 23-24.	22, 23, 24.
			June 4-5.	June 5-6.	June 6-7.	25, 26, 27. ^b

* Off feed on Mar. 2 to 4, inclusive.

^b Fourth, fifth, and sixth days of fast.

Each experimental period consisted of a preliminary feeding period at least 7 days in length, a period of 10 to 12 days' duration in which the feces and urine were collected for analysis, and 3 days, consecutive or intermittent, in the respiration chamber. The amount of feed consumed was kept constant throughout each experimental period. These statements do not apply entirely to the period on full feed, since it was found difficult to keep the steer continuously on this high intake. The collection period in this case was of necessity reduced to 5 days, and the last day in the respiration chamber was marred by the refusal of part of the ration.

The collection periods in these experiments are shorter than those at times employed in metabolism work with cattle. At the Institute of Animal Nutrition of the Pennsylvania State College, for example, collection periods of 17 to 21 days are commonly used, while Møllgaard (17) advocates even longer periods. A 7-day preliminary feeding period is also considered inadequate by this investigator, although in the recently published experiments of Forbes and his associates, the preliminary feeding periods were frequently as short as 4 or 5 days. Judging by the results obtained at this laboratory there appears to be

no necessity for long preliminary periods or long collection periods in evaluating the digestibility or the metabolizable energy of rations for cattle, although in the evaluation of nitrogen or mineral balances characteristic of a given nutritive régime the necessity for longer periods seems much greater.

The speed with which the digestive tract of the ruminant adjusts itself to a new feed mixture is indicated by some digestion experiments performed in this laboratory on two sheep and four steers, carried on according to the chromic oxide method of Hedin or a modified method in which ferric oxide was taken as the inert material. The two sheep were receiving daily in two feedings 2 pounds of chopped alfalfa hay. After a number of days on hay alone, each sheep was given in addition 1.585 g of chromic acid, dispersed in an agar-agar jelly and divided equally between the two feedings. The feces were collected daily and analyzed for dry matter and chromium. Chromium appeared in the feces of the first collection and increased in concentration up to the fourth day; from this time on the ratio of chromium to fecal dry matter maintained a level, the dispersion of the ratios for individual days around the mean of 18 days being expressed by coefficients of variation of 7.13 and 7.84. At the end of 18 days of feeding, the chromic oxide dosage was discontinued, and on the third day thereafter chromium had practically disappeared from the feces, the concentration decreasing from 0.222 to 0.006 per cent in one case and from 0.174 to 0.0004 per cent in the other. The experiments with steers gave essentially the same results, i. e., a rapid attainment of a fairly constant ratio of fecal dry matter to inert metal, a maintenance of this ratio as long as the inert material was fed with a constancy of 6 to 10 per cent (average 7.5 per cent) expressed as the coefficient of variation, and a rapid disappearance of the inert metal from the feces following its removal from the ration.

TABLE 2.—Comparison of digestion coefficients obtained in 14-day and in 3-day collection periods on alfalfa and on timothy hay

ALFALFA HAY						
Steer No.	Length of collection period days	Coefficients of digestibility				
		Dry matter	Crude protein	N-free extract	Crude fiber	Ether extract
1.....	14	59	70	66	52	60
	3	59	69	66	53	52
2.....	14	54	65	62	47	-2
	3	56	67	65	45	40
3.....	14	59	67	67	54	54
	3	60	69	66	55	45
4.....	14	56	69	65	46	26
	3	51	68	58	42	0
Averages.....	14	57.0	67.8	65.0	49.8	34.5
	3	56.5	68.3	63.8	48.8	34.3
TIMOTHY HAY						
1.....	14	50	12	56	53	26
	3	50	14	56	53	33
2.....	14	58	40	64	61	50
	3	56	39	62	58	53
4.....	14	54	46	59	53	52
	3	53	47	50	52	54
Averages.....	14	54.0	32.7	59.7	55.7	42.7
	3	53.0	33.3	56.0	54.3	46.7

Evidence of the same significance was obtained by the use of the ordinary method of determining digestibility. Four steers were fed in trials on alfalfa hay and three in trials on timothy hay. In each trial there was a preliminary period of 14 days followed by a collection period of 14 days. The feces of the first 3 days were aliquoted for incorporation into two samples, one for 3 days and the other for the full 14 days, and coefficients for both periods were determined. The results are assembled in Table 2.

For two steers on each hay, complete data were obtained for the computation of the metabolizable energy in the 3-day and 14-day collection periods, with the exception of methane excretion, which was estimated by the use of Armsby's factor of 4.5 g of methane per 100 g of digestible carbohydrates consumed. For the alfalfa hay the values in calories per kilogram of dry matter were 2,103 and 2,066 for the 14-day period, and 2,075 and 2,130, for the 3-day period, representing differences of -1.35 and +3.00 per cent. For the timothy hay the values were 1,858 and 1,824 for the 14-day periods and 1,849 and 1,776 for the 3-day periods, the differences being -0.49 and -2.70 per cent.

These data are presented, not with the idea of advocating such a short collection period as 3 days in digestion trials with steers, but in support of the general proposition that the need for long periods of 20 days or more is probably greatly exaggerated. The 10-day and 14-day periods used in the experiments reported below were long enough, the writers believe, to give accurate determinations of the metabolizable energy content of the rations, especially in view of the fact that examination of the daily records of fecal excretion revealed no marked irregularities at the beginning and end of the periods. The 5-day collection period on the highest level of feeding, necessitated by the refusal of the steer to consume this heavy ration for a longer time, might well be questioned were it not for the remarkable regularity in fecal excretion that obtained throughout. The daily weights of fresh feces during this period were 12.21, 12.05, 12.81, 14.24, and 12.38 kg, averaging 12.74 kg.

The respiration chamber used in these experiments (fig. 1) is of the open-circuit type. The principle of its construction and operation is the same as that of the chamber at the New Hampshire Agricultural Experiment Station described in bulletin 240 of that station (19), and of several respiration chambers used in the study of human metabolism at the nutrition laboratory of the Carnegie Institution of Washington, located at Boston. The outgoing air is aliquoted according to the Benedict principle, the size of aliquot being determined by the size of disk through which the main portion of the outgoing air passes from the apparatus. The size of aliquot obtained with each disk is carefully standardized by introducing pure carbon dioxide into the wind chest and determining the amount recovered from the sampling cans. The aliquoting table (fig. 2) accommodates 2 trains of bottles below for the absorption of water, 4 trains of bottles for the absorption of carbon dioxide, and 2 dry gas meters to measure the volume of the samples. This arrangement permits the taking of two simultaneous samples of chamber air. In this series of experiments the average difference between the simultaneous 24-hour samples of CO_2 was 1.16 per cent, 65 per cent of the

differences being less than 1 per cent and 85 per cent being less than 2 per cent.

The Illinois apparatus differs from the New Hampshire apparatus mainly in the construction of the chamber. In the Illinois apparatus

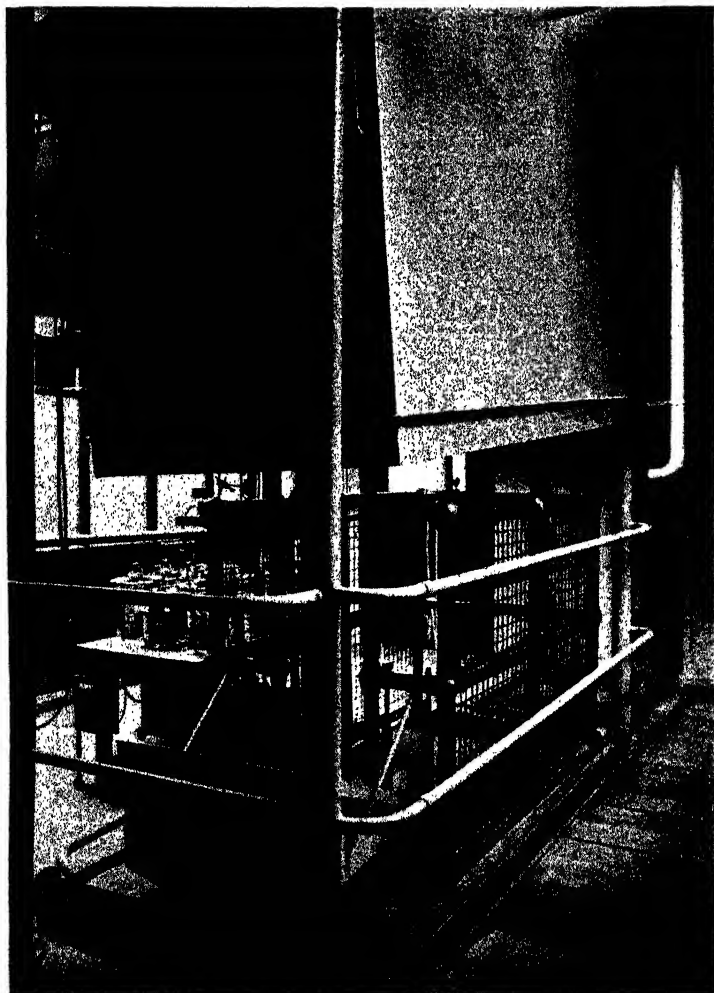


FIGURE 1.—General view of respiration chamber with cover up

the chamber consists of a base supporting a heavy iron stall framework, with a stanchion and a manger, containing a water trough which may be filled from the outside. A water seal completely surrounds the base of the chamber, and receives the heavy metal box constituting the walls and ceiling of the chamber and containing at the front an opening guarded by a cover sunk in a water seal

through which feed may be dumped into the manger. To admit the steer the metal box is raised to the ceiling of the room 14 feet from the floor by a system of ropes and pulleys, the process being facilitated by counterweights concealed in the four metal posts located at the four corners of the chamber. The reverse process secures the steer in an air-tight chamber from which connections are made with the incoming air pipe leading through the roof of the room to the outside, and with the outgoing air pipe leading to the wind chest on the aliquoting table. The ventilating fan is located at the exit opening, while another smaller fan at the upper front end of the metal box circulates the air in the chamber and prevents the formation of a

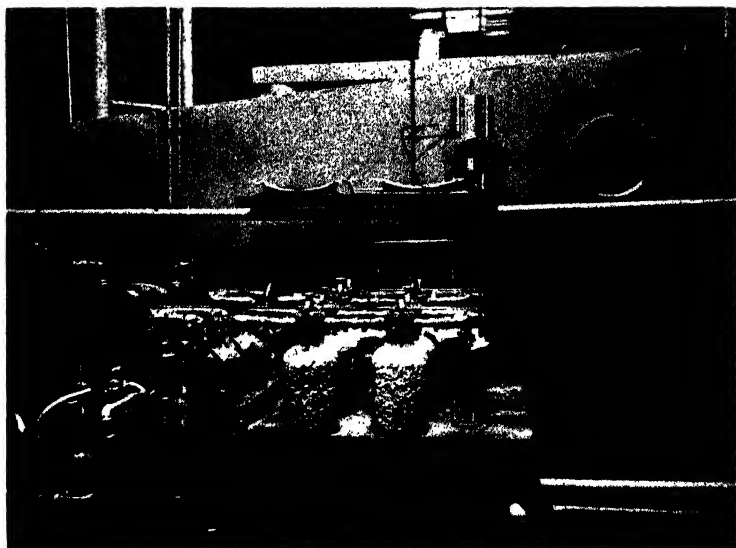


FIGURE 2.—Aliquoting table used with respiration chamber

dead air pocket. The volume of the chamber when containing a 1,000-pound steer is 6,581 liters.

The chamber is lighted electrically and contains a thermometer, a hygrometer, and a work adder attached to the movable platform on which the front feet of the steer rest. All of these instruments may be read from the glass window in the side. A pneumograph attached to the movable platform and extending through the base to the necessary recording arrangements supported on a shelf over the aliquoting table, permits the taking of a graphic record of the up and down excursions of this platform, measuring, albeit roughly, the activity of the steer. The chamber also contains an automatic device that rings a bell whenever the steer changes his position from standing to lying or the reverse.

The base of the chamber contains openings for the collection of feces and urine in a basement beneath, the former in a metal container suspended from the base and connected with it through a water seal, the latter in a brass cylinder into which the urine hose extends and from which the urine flows, through a water seal, into a glass carboy.

The apparatus itself permits of the continuous determination of the carbon dioxide produced by the steer. This was effected in six 4-hour subperiods. In order to determine the respiratory quotient, a continuous sample of air was taken at a constant rate from the outgoing air pipe and stored for each 4-hour subperiod in a gasometer with a capacity of approximately 100 l. Each sample collected in this fashion was then analyzed for carbon dioxide and oxygen in a Carpenter-Haldane analyzer (8). In addition, samples of air were taken during a 10-minute interval at the start of the experiment and at the end of each subperiod in order to determine the carbon dioxide content of the chamber to be used in computing the CO₂ production for the subperiods. The gas analyzers were checked each day by analyses of outdoor air and were not used until the results obtained fell within the limits 0.030 ± 0.003 for CO₂ and 20.940 ± 0.005 for O₂.

TABLE 3.—The ratio of methane produced to digestible carbohydrates consumed by steers, at various levels of feeding, from the published experimental data of the Institute of Animal Nutrition, Pennsylvania State College

Steer No.	Period No.	Ration	Plane of nutrition	Methane produced	Methane per 100 g. digestible carbohydrates
				Grams	
60	1	Alfalfa + corn	Maintenance	79.5	4.51
57	2			95.3	4.96
47	7			108.6	4.61
36	8			111.0	4.76
57	12	119.4		5.14	
60	13	Alfalfa alone		109.8	4.95
47	9			125.7	5.03
36	10			124.1	4.96
Average					4.86
60	3	Alfalfa + corn	1.5 maintenance	111.4	4.32
57	4			133.5	4.60
47	3			140.9	4.13
36	4			138.0	4.24
Average					4.32
60	5	Alfalfa + corn	2.0 maintenance	156.3	4.54
57	6			175.0	4.57
47	1			183.2	4.19
36	2			162.5	3.92
Average					4.30
60	7	Alfalfa + corn	2.5 maintenance	188.7	4.19
57	8			219.6	4.56
Average					4.37
60	9	Alfalfa + corn	3.0 maintenance	230.4	4.22
47	5	do	0.5 maintenance	62.0	5.41
36	6			62.3	5.42
57	10			55.8	5.30
60	11			55.3	5.36
Average					5.37

In each respiration experiment the steer was put into the chamber at 8 a. m., at which time one-half of the day's feed was put before him in the manger. The ventilation was not started until 8.15, in order that the carbon dioxide content of the chamber might reach, approximately, the level maintained throughout the day. When the ventilation was started a sample of air was taken for analysis of CO₂. The other half of the day's feed was given at 4 p. m. The steer was given measured amounts of water after each feeding.

During the entire experiment two men were always in attendance, and every half hour they recorded the temperature and humidity in the chamber, the position of the steer, the reading of the work adder, and the reading of the gas meters.

The methane content of the outgoing air was not determined. In the computation of the metabolizable energy content of the rations, the true carbon dioxide and oxygen content of the chamber air, and the heat production of the steer, the production of methane was estimated from the intake of digestible carbohydrates. Arnsby has recommended a factor of 4.5 g of CH_4 per 100 g of digestible carbohydrates. However, in view of the fact that the level of feeding differed widely in the different experimental periods and that the methane production per unit of digestible carbohydrates may be presumed to vary with the level of feeding, especially at low levels, the relation of this factor to the level of feeding was investigated from the recently published experimental data of Forbes and his associates (9, 10). In Table 3, computations of the ratio of methane produced to digestible carbohydrates consumed, made from the Pennsylvania data, have been summarized.

It appears from the computations that the ratio is not subject to any appreciable change from levels of feeding ranging from 1.5 to 3.0 times the maintenance level. At the lower levels of feeding, however, the ratio increased considerably. Therefore, for the periods in the present experiment involving full feed, four-fifths feed, and three-fifths feed, the writers have continued to use the factor of 4.5 g of methane per 100 g of digestible carbohydrates in preference to a lower factor of 4.3, favored by the data in Table 3, since it is based upon somewhat more data and since the difference between the two factors is inappreciable in its effect on the calculations in this experiment. Since the two-fifths full feed level proved to be close to a maintenance ration, a factor of 4.85 g of methane per 100 g of digestible carbohydrates was used in this period, and a factor of 5.5 g was used in the one-fifth full feed period.

RESULTS OF THE EXPERIMENT

THE BODY WEIGHTS OF THE STEER

The body weights of the steer at the beginning and end of each collection period and at the time of each confinement in the respiration chamber are given in Table 4. Each weight given is the average for three consecutive days. When respiration experiments were undertaken on three consecutive days, only two average weights are given, one for the morning on which the steer entered the chamber and the two preceding mornings, and the other for the morning on which the steer left the chamber and the two following mornings.

TABLE 4.—*Body weights of the steer in kilograms at beginning and end of each collection period and at time of each confinement in the respiration chamber*

Plane of nutrition	Collection period		Respiration experiments		
	Initial weight *	Final weight *	First weight *	Second weight *	Third weight *
Four-fifths full feed	582.0	594.2	591.5	590.1	597.4
Full feed	611.5	622.8	621.4	625.5	622.3
Three-fifths full feed	624.2	627.3	624.6	632.8	627.8
Fasting			632.3		623.7
Two-fifths full feed	610.1	615.1	614.6	607.8	615.1
One-fifth full feed	604.6	588.8	604.6		599.7
Fasting			603.3		602.4

* Each weight given represents an average for 3 consecutive days.

THE DIGESTIBILITY OF THE RATION AT DIFFERENT LEVELS OF FEEDING

The complete data relating to the calculation of the digestion coefficients of dry substance, crude protein, nitrogen-free extract, crude fiber, and ether extract are assembled in Table 5. To facilitate comparison, the coefficients have been summarized in Table 6. Table 7 gives the average composition of the feeds used.

TABLE 5.—*The calculation of digestion coefficients of various ration constituents at different levels of feeding*

FULL FEED

Item	Direct weight	Dry substance	Crude protein	N-free extract	Crude fiber	Ether extract
Alfalfa.....grams	3,000	2,761	429	1,215	786	105
Corn.....do	10,000	8,159	787	6,663	223	363
Linseed meal.....do	250	228	92	91	20	11
Molasses.....do	116	81	5	66		
Total.....do		11,229	1,313	8,035	1,029	479
Feces.....do	12,739	2,954	463	1,572	512	190
Nutrients digested.....do		8,275	850	6,463	517	289
Digestibility.....per cent		73.7	64.7	80.4	50.2	60.3

FOUR-FIFTHS FULL FEED

Alfalfa.....grams	2,400	2,209	335	966	624	58
Corn.....do	8,000	6,514	655	5,250	243	266
Linseed meal.....do	200	182	73	72	17	10
Molasses.....do	100	70	5	57		
Total.....do		8,975	1,068	6,345	884	334
Feces.....do	10,879	2,300	409	1,102	506	101
Nutrients digested.....do		6,675	659	5,243	378	233
Digestibility.....per cent		74.4	61.7	82.6	42.8	69.8

THREE-FIFTHS FULL FEED

Alfalfa.....grams	1,800	1,627	244	694	507	50
Corn.....do	6,000	4,990	465	4,100	151	205
Linseed meal.....do	150	136	55	55	12	8
Molasses.....do	75	53	3	43		
Total.....do		6,806	767	4,892	670	263
Feces.....do	7,440	1,552	315	709	306	72
Nutrients digested.....do		5,254	452	4,183	364	191
Digestibility.....per cent		77.2	58.9	85.5	54.3	72.6

TWO-FIFTHS FULL FEED

Alfalfa.....grams	1,200	1,115	172	482	318	45
Corn.....do	4,000	3,402	320	2,753	120	154
Linseed meal.....do	100	92	36	37	8	5
Molasses.....do	50	35	2	29		
Total.....do		4,644	530	3,301	446	204
Feces.....do	4,167	888	196	336	212	40
Nutrients digested.....do		3,756	334	2,965	234	164
Digestibility.....per cent		80.9	63.0	89.8	52.5	80.4

TABLE 5.—*The calculation of digestion coefficients of various ration constituents at different levels of feeding—Continued*

ONE-FIFTH FULL FEED

Item	Direct weight	Dry substance	Crude protein	N-free extract	Crude fiber	Ether extract
Alfalfa.....grams.	600	549	96	229	154	13
Corn.....do	2,000	1,710	156	1,386	65	76
Linseed meal.....do	50	46	18	19	4	2
Molasses.....do	25	18	1	14		
Total.....do		2,323	271	1,648	223	91
Feces.....do	1,738	373	87	121	86	14
Nutrients digested.....do		1,950	184	1,527	137	77
Digestibility.....per cent.		83.9	67.9	92.7	61.4	84.6

TABLE 6.—*Summary of digestion coefficients (per cent) of various ration constituents at different levels of feeding*

Plane of nutrition	Dry substance	Crude protein	N-free extract	Crude fiber	Ether extract
Full feed.....	73.7	64.7	80.4	50.2	60.3
Four-fifths full feed.....	74.4	61.7	82.6	42.8	69.8
Three-fifths full feed.....	77.2	58.9	85.5	54.3	72.6
Two-fifths full feed.....	80.9	63.0	89.8	52.5	80.4
One-fifth full feed.....	83.9	67.9	92.7	61.4	84.6

TABLE 7.—*Average percentage chemical composition of feeds used*

Feed	Dry substance	Crude protein	N-free extract	Crude fiber	Ether extract	Ash	Gross energy
							<i>Calories per gram</i>
Alfalfa hay.....	91.79	14.42	39.53	26.52	2.94	8.37	3.97
Corn.....	83.35	7.92	67.75	2.80	3.60	1.28	3.08
Linseed meal.....	91.23	36.49	36.81	7.93	4.81	5.20	4.31
Molasses.....	70.26	4.51	57.13			6.62	2.64

The lowest level of feeding was associated with the most complete digestibility of all nutrients. However, there was a progressive decrease in digestibility from the lowest to the highest ration only in the case of nitrogen-free extract, ether extract, and dry substance.

THE METABOLIZABLE ENERGY AT DIFFERENT LEVELS OF FEEDING

The computation of the total metabolizable energy consumed at the different levels of feeding is given in Table 8. The usual correction of the energy loss in the urine to a condition of nitrogen equilibrium has been made on the assumption that each gram of nitrogen retained by (or lost from) the body would on excretion in the urine (or retention in the body) carry with it 7.45 calories of nonmetabolizable energy. The metabolizable energy values thus obtained more nearly represent the actual content of metabolizable energy in the rations consumed. The nitrogen balances upon which this correction is based are given in Table 9.

TABLE 8.—*Calculation of the metabolizable energy, in Calories, of the ration at different levels of feeding*

Plane of nutrition	Gross energy of feeds consumed					Energy of feces	Energy of urine			Energy of methane	Total metabolizable energy
	Alfalfa hay	Corn	Linseed meal	Molasses	Total		Observed	Correction to nitrogen equilibrium *	Corrected		
Full feed	12,030	35,880	1,072	307	49,295	14,038	861	+326	1,187	4,189	29,881
Four-fifths full feed	9,708	28,896	849	264	39,717	11,020	729	+273	1,002	3,375	24,320
Three-fifths full feed	6,934	21,876	650	198	29,658	7,343	548	+56	604	2,720	18,982
Two-fifths full feed	4,808	15,032	436	132	20,406	4,209	503	+64	567	2,069	13,563
One-fifth full feed	2,368	7,598	216	66	10,248	1,594	319	-42	277	1,220	7,187

* See nitrogen balances given in Table 9.

TABLE 9.—*The daily nitrogen balances (grams) of the steer at different levels of feeding*

Plane of nutrition	Nitrogen in feed	Nitrogen in feces	Nitrogen in urine	Nitrogen excreted	Nitrogen balance
Full feed	210.21	74.19	92.31	166.50	+43.71
Four-fifths full feed	170.78	65.45	68.72	134.17	+36.61
Three-fifths full feed	122.75	50.43	64.59	115.02	+7.73
Two-fifths full feed	84.86	31.41	44.84	76.25	+8.61
One-fifth full feed	43.41	13.98	35.02	49.00	-5.59

A further study of the losses of nonmetabolizable energy and of the metabolizable energy remaining is permitted from the data summarized in Table 10. In particular, it is important to note that the metabolizable energy per kilogram of dry matter consumed, as well as the percentage metabolizability of the gross energy, increases progressively from the highest to the lowest level of feeding, the former from 2,661 Calories to 3,094 Calories, and the latter from 60.61 per cent to 70.14 per cent. However, when the metabolizable energy is computed to a kilogram of digestible nutrients, all effect of the level of feeding disappears.

TABLE 10.—*Further computations with reference to the metabolizable energy of rations*

Plane of nutrition	Dry matter eaten daily	Digestible nutrients eaten daily	Energy per kilogram of dry matter						Losses of energy			
			Total	Losses			Metabolizable	Metabolizable energy per kilogram of total digestible nutrients	In feces	In urine	In methane	Metabolizable energy
				In feces	In urine	In methane						
	Kilograms	Kilograms	Calories	Calories	Calories	Calories	Calories	Calories	Per cent	Per cent	Per cent	Per cent
Full feed	11.229	8.490	4,390	1,250	106	373	2,661	3,524	28.47	2.41	8.50	60.61
Four-fifths full feed	8.975	6.002	4,425	1,229	112	376	2,709	3,578	27.75	2.53	8.50	61.22
Three-fifths full feed	6.806	4.426	4,358	1,074	88	401	2,795	3,498	24.64	2.02	9.20	64.13
Two-fifths full feed	4.544	3.599	4,394	906	122	446	2,920	3,478	20.62	2.78	10.15	66.45
One-fifth full feed	2.323	2.022	4,411	673	119	525	3,064	3,564	15.26	2.70	11.90	70.14

In view of the fact that the methane production was estimated, not directly determined, in this experiment, it is worthy of note that the energy lost as methane amounted to only 8.5 to 11.9 per cent of the gross energy consumed. Hence, even a considerable percentage error in the estimation of the methane production may exert only an inappreciable error on the computation of the metabolizable energy values.

THE ENERGY METABOLISM OF THE STEER

The results of the respiration experiments have been assembled in Table 11. The average respiratory quotients have been obtained from the average gas analyses of air samples collected in the six subperiods, after correction for the estimated methane content of the chamber air. The volume of oxygen consumed was obtained from the volume of carbon dioxide produced and the respiratory quotient.

TABLE 11.—*The results of the respiration experiments*

Date of experiment	Live weight of steer	Dry matter consumed	Chamber temperature		Chamber humidity		Hours standing out of 24	Average respiratory quotient	Carbon dioxide produced		Oxygen consumed
			Average	Range	Average	Range			Grams	Liters	
	Kilo-grams	Grams	° C.	° C.	Per cent	Per cent					Liters
Feb. 28-29	621.4	11,229	22.9	21.5-25.0			14.33	1.16	8,692	4,425	3,815
Mar. 1-2	625.5	11,229	21.6	21.0-22.2	93	81-98	12.13	1.12	8,194	4,172	3,725
Mar. 9-10	622.3	11,229	21.1	18.9-22.2	95	90-98	13.72	1.11	7,863	4,003	3,606
Feb. 1-2	591.5	8,976	23.7	22.5-24.5			16.55	1.14	7,242	3,687	3,234
Feb. 3-4	586.1	8,976	24.5	23.0-24.8			12.82	1.11	6,786	3,455	3,113
Feb. 13-14	597.4	8,976	22.8	21.8-23.5			15.47	1.12	7,051	3,590	3,205
Mar. 30-31	624.6	6,807	18.6	17.2-20.0	89	77-99	16.85	1.03	6,137	3,124	3,033
Apr. 2-3	632.8	6,807	23.1	21.6-25.0	93	87-97	8.27	1.08	5,789	2,947	2,729
Apr. 5-6	627.3	6,807	22.8	22.2-23.3	95	89-99	12.87	1.04	6,124	3,118	2,998
May 2-3	614.6	4,644	22.8	21.6-25.5	83	71-92	11.38	.95	4,745	2,416	2,543
May 8-9	607.8	4,644	22.8	21.1-25.6	76	65-87	11.35	.97	4,887	2,488	2,565
May 9-10	615.1	4,644	23.9	22.2-26.7	87	78-93	11.98	.98	4,968	2,529	2,581
May 21-22	604.6	2,323	25.6	21.8-27.2	86	83-90	13.60	.87	3,574	1,820	2,092
May 22-23		2,323	23.3	21.6-25.0	85	81-88	11.10	.88	3,436	1,749	1,982
May 23-24	599.7	2,323	22.8	21.1-25.0	80	79-84	11.35	.89	3,491	1,777	1,997
Apr. 10-17	632.3	0	20.0	17.2-21.6	72	66-78	12.43	.70	2,887	1,470	2,100
Apr. 17-18		0	20.6	20.0-21.1	93	90-96	9.03	.71	2,801	1,426	2,008
Apr. 18-19	623.7	0	21.6	20.6-22.8	91	89-94	9.35	.71	2,761	1,406	1,980
June 4-5	603.3	0	20.6	20.0-21.1	97	94-100	12.68	.69	2,765	1,408	2,011
June 5-6		0	21.1	20.6-22.8	95	91-97	12.62	.68	2,711	1,380	1,971
June 6-7	602.4	0	21.1	20.0-21.6	95	92-98	13.30	.68	2,717	1,383	1,976

* Considerable feed was refused on this day.

In Table 12 the respiratory quotients of the six subperiods in each experiment are given. The gas analyses at the lowest plane of nutrition were in error in a large portion of the cases, although at least one good respiratory quotient was available for each experimental subperiod of four hours. The average respiratory quotient for the day at this level was estimated as accurately as possible from the reliable analyses available, supplemented by information concerning the CO₂ content of the chamber air obtained from the continuous gravimetric determination of carbon dioxide on the aliquoting table and the total volume of air withdrawn from the chamber.

It is evident from the values given in Table 12 that (1) at the two highest levels of feeding the respiratory quotient was continuously greater than 1, (2) at the level of three-fifths full feed the quotient was above 1 for approximately eight hours after each feeding, (3) at the level of two-fifths full feed a quotient greater than unity did not obtain for more than four hours after feeding, and (4) at the lowest

level of feeding no quotients greater than unity were observed, although such quotients may have obtained for a short period after feeding. At all levels of feeding except the lowest, the respiratory quotient for the first four hours after feeding was higher than for any other period, and progressively decreased at the higher levels of feeding, less clearly at levels lower than these.

TABLE 12.—*The respiratory quotients obtained during the six subperiods in each experiment, together with the average quotients for the day*

Plane of nutrition	Experiment No.	Respiratory quotient during indicated hours after feeding						Total respiratory quotients for the day		
		1 to 4	4 to 8	1 to 4	4 to 8	8 to 12	12 to 16	Observed	Corrected for methane ^a	Corrected to complete oxidation ^b
Full feed.....	{ 10	1.19	1.13	1.22	1.09	1.08	1.04	1.13	1.16	1.05
	{ 11	1.13	1.04	1.13	1.10	1.08	1.02	1.08	1.12	1.01
	{ 12	1.06	1.09	1.10	1.09	1.03	1.00	1.08	1.11	1.00
Average.....		1.13	1.09	1.15	1.09	1.06	1.05	1.10	1.13	1.02
Four-fifths full feed.....	{ 7	1.16	1.06	1.14	1.10	1.05	1.10	1.11	1.14	1.03
	{ 8	1.16	1.04	1.20	1.04	1.05	1.00	1.08	1.11	1.01
	{ 9	1.13	1.08	1.21	1.06	1.05	.99	1.09	1.12	1.02
Average.....		1.15	1.07	1.18	1.07	1.05	1.03	1.09	1.12	1.02
Three-fifths full feed.....	{ 13	.97	1.05	1.12	.99	.96	.97	1.01	1.03	.96
	{ 14	1.12	1.01	1.17	1.00	1.04	.95	1.05	1.08	.99
	{ 15	1.01	1.04	1.09	1.03	.98	.96	1.02	1.04	.96
Average.....		1.03	1.03	1.13	1.01	.99	.96	1.03	1.05	.97
Two-fifths full feed.....	{ 19	.95	.99	---	.90	.85	.96	.93	.95	.89
	{ 20	.92	.98	1.10	.85	.88	.95	.95	.97	.91
	{ 21	1.17	.92	1.03	.89	.90	.80	.96	.98	.92
Average.....		1.01	.96	1.06	.88	.88	.93	.95	.97	.91
One-fifth full feed.....	{ 22	.87	.87	---	.90	.90	---	.86	.87	.84
	{ 23	.87	---	---	.91	.83	---	.87	.88	.84
	{ 24	---	---	.93	.95	---	.81	.87	.89	.85
Average.....		.87	.87	.91	.92	.83	.81	.87	.88	.84

^a Gas analyses corrected for presence of methane.

^b Assuming that the methane had been oxidized to CO₂ and H₂O.

The average respiratory quotients for the 24 hours were, in the order of decreasing levels of feeding, 1.10, 1.09, 1.03, 0.95, and 0.87. However, these quotients are to some extent in error, because of the methane production. The oxygen deficit in the chamber air can not be estimated accurately in the presence of appreciable quantities of methane without making due allowance for the latter; the CO₂ excess in the chamber air is not appreciably affected by the presence of methane in the concentration produced. From the estimated day's production of methane, based as explained above on the intake of digestible carbohydrates, the following volumes of methane produced for each liter of CO₂ were estimated, in the order of decreasing levels of feed intake, i. e., 0.0932, 0.0881, 0.0831, 0.0779, and 0.0639 liters. The average percentages of CO₂ in the expired air for 24 hours were, in the same order, 0.614, 0.534, 0.455, 0.387, and 0.305. From these two types of data the average percentages of methane in the chamber were estimated to be as follows, in the order of decreasing levels of feeding: 0.056, 0.046, 0.038, 0.030, and 0.019. The methane correction of the observed gas analysis values diminished the uncorrected

oxygen deficit in the expired air and increased the average respiratory quotients from the average values given above to the following average values, given in the usual order: 1.13, 1.12, 1.05, 0.97, and 0.88.

However, these respiratory quotients are not usable in the computation of heat production, since they do not correspond to complete oxidation of the food nutrients. To obtain usable respiratory quotients, the observed volumes of CO_2 produced and O_2 consumed must be increased by the volumes of these gases involved in the complete oxidation of the amounts of CH_4 estimated to have been produced. This has been done in computing the respiratory quotients given in the last column of Table 12. The heat production per 24 hours was estimated from the volume of oxygen consumed (Table 11) plus the volume of oxygen required to oxidize the methane formed. If the average respiratory quotient for the day, computed with reference to complete oxidation, was above unity, the heat equivalent of the oxygen was taken as 5.047 Calories per liter, the value for a respiratory quotient of unity. To allow for the heat evolved in the conversion of carbohydrates to fats, as indicated by a respiratory quotient above unity, the excess of CO_2 produced over the O_2 consumed was considered to represent an extra heat output of 0.803 Calorie per liter,³ a factor that Lusk has computed from the equation of Bleibtren for the conversion of glucose to fat. The heat production for the day was considered to be given, following the method of Anderson (1), by the sum of these two quantities of heat minus the heat of combustion of the methane produced. If the average respiratory quotient for the day was less than unity, the heat equivalent of the oxygen consumed plus that required to oxidize the methane was determined from the well-known tables of Lusk (14), and from the heat thus computed was deducted the heat equivalent of the methane.

It is interesting to consider the effect of these corrections for the production of methane and for its presence in the chamber air upon the computation of heat production. The average percentage errors incurred by a neglect of these corrections would be, for the different levels of feeding, +5.35, +4.87, +5.08, +5.63, and +2.04. The two corrections above described are of about equal importance in their effect upon the final computations.

In the method of computation just described the protein metabolism is entirely neglected, the total respiratory quotients being given the significance of nonprotein respiratory quotients in the determination of the caloric equivalent of each liter of oxygen consumed. There is a method in somewhat common use for making allowance for protein in these calculations by the use of certain correction factors, by which the CO_2 and heat produced in protein metabolism and the oxygen consumed are estimated from the nitrogen excreted in the urine. By using these factors, a nonprotein respiratory quotient may be computed in each respiration experiment.

³ In the fourth edition of Lusk's book *The Science of Nutrition* (14, p. 396) a recalculation of this factor has been made, leading to a value of 1.09 calories per liter of CO_2 produced in the transformation of carbohydrate to fat. The writers' attention was called to this revision after all the calculations given in this paper were completed. When it was ascertained that the new factor would occasion an entirely insignificant change in the values for total heat production, less than 100 calories and generally considerably less, the task of making this revision was not undertaken. This method of evaluating the heat produced in the conversion of sugar to fat is based upon a hypothetical equation of Bleibtren, and its validity is not universally admitted among investigators in animal and human calorimetry. It is an attempt to evaluate the maximum production of fat from sugar, and a number of other equations, involving different energy relations, have been proposed. It seems impossible to select the most probable equation for this transformation on the basis of any accurate information available.

The derivation of these factors is described in considerable detail by Lusk (14), and his factors are used rather widely in this country. It is a significant fact that they are based upon the composition of meat protein and upon the extent of its metabolizability in the dog. They are applied, however, not only to fasting animals, in which the protein katabolism relates mainly to muscle protein oxidation, but to animals on rations containing any variety of protein. In these cases the protein katabolism relates to dietary protein, and the relations of respiratory CO_2 and O_2 and of heat to the urinary nitrogen must be different from those calculated for meat protein. To apply meat-protein factors to such conditions is to assume that all proteins will give essentially the same factors, although it is known that the nitrogen content of vegetable proteins may vary considerably from that of meat protein. The relative indigestibility of vegetable proteins would complicate further the computation of factors such as those devised by Lusk for meat protein.

Magnus-Levy (15) has expanded upon this difficulty in the way of an accurate assessment of the share of protein in the respiratory metabolism of animals and has compromised by giving factors for protein which are the averages of values relating to "muscle substance" and to casein. According to Lusk, 1 liter of oxygen used in the combustion of protein is equivalent to 4.463 Calories of heat; according to Magnus-Levy it is equivalent to 4.578 Calories. Benedict and Joslin (5) have computed a value of 4.60 Calories for this factor, while Møllgaard's factors (17) lead to a value of 4.639 Calories. According to Lusk, each gram of nitrogen excreted in the urine represents 26.51 Calories of protein metabolism; according to Magnus-Levy, 27.14 Calories; and according to Møllgaard, 28.12 Calories.

It is evidently impossible to assess accurately the extent of the participation of protein in the respiratory and energy metabolism of animals from the excretion of nitrogen in the urine, and fortunately the need for this computation is not great, since the error consequent upon its neglect will never, under practical conditions of feeding, be considerable. This fact depends upon the further fact that the heat value of a liter of oxygen used in the combustion of protein appears to differ but slightly from the heat value of a liter of oxygen used in the oxidation of a mixture of fat and sugar having the same respiratory quotient as protein, i. e., 4.803 to 4.810, depending upon the precise respiratory quotient assigned to protein. The maximum error would obviously be made when the total observed respiratory quotient is 0.802 and when protein is the only nutrient undergoing oxidation, a condition theoretically possible but rarely if ever realized in actual nutrition. If, now, protein metabolism be entirely neglected and the total respiratory quotient be taken as a nonprotein respiratory quotient, then the error in the estimation of the heat value of each liter of oxygen consumed would, according to the values accepted by Lusk, be $4.803 - 4.463 = 0.340$ Calories, or 7.62 per cent. If Benedict's value for protein is taken, the maximum error would be $4.803 - 4.600 = 0.203$ Calories, or 4.41 per cent, while, if Møllgaard's value is used, an error of $4.803 - 4.639 = 0.164$ Calories, or 3.54 per cent would result.

This is evidently an extreme condition, since the protein metabolism for the day rarely exceeds 20 to 25 per cent of the total, and, according to Magnus-Levy, is generally no greater than 15 per cent

of the total. Also as the total respiratory quotient deviates from 0.80 the maximum possible protein metabolism rapidly decreases, as well as the maximum error realized by its neglect. The calculations in Table 13 illustrate this fact. Thus, for a total respiratory quotient of 0.95, it would be possible, in the event of no oxidation of fats, for 23 per cent of the heat produced to result from the oxidation of protein. However, if this unusual condition actually existed and if the computation of the heat production were made on the assumption that the total respiratory quotient was a nonprotein respiratory quotient, the result would be in error by only 1.7 per cent.

TABLE 13.—*The maximum error made in neglecting protein metabolism in the computation of the heat production from the respiratory exchange*

Total respiratory quotient	Maximum protein metabolism possible	Heat value of a liter of oxygen for maximum protein metabolism	Heat value of a liter of oxygen, neglecting protein metabolism	Maximum error in neglecting protein metabolism
	Per cent	Calories	Calories	Per cent
0.75.....	45	4.584	4.739	3.3
0.80.....	100	4.463	4.801	7.6
0.85.....	73	4.607	4.863	5.6
0.90.....	47	4.754	4.924	3.6
0.95.....	23	4.901	4.985	1.7

It would be extremely unlikely under any conditions that the maximum percentage of protein metabolism would ever be realized. If no more than 20 per cent of the total heat produced is derived from the oxidation of protein, a fairly high level, the maximum error committed by neglecting protein metabolism entirely would be about 1.5 per cent. In the present experiment the protein calories constitute less than 13 per cent of the total calories, as ordinarily computed from the nitrogen in the urine (Table 9), and the differences between the total heat production computed with and without a correction for protein metabolism, by using Lusk's factors, range from 1.25 per cent at the level of full feed to 0.69 per cent at the level of one-fifth full feed. It is true that it would be advisable to avoid errors even of this magnitude if a good method of doing so were at hand. It is probable that the uncorrected values are slightly too high, but there is no assurance that if Lusk's factors were used in correcting the heat production for protein metabolism the estimates obtained would not be slightly too low.

In view of the uncertainty in all methods proposed for the measurement of the participation of protein in the respiratory and energy metabolism of animals, and in view of the inconsiderable error that results apparently from the entire neglect of this point, the writers prefer to make their indirect calorimetric computations on the basis of total respiratory quotients rather than nonprotein respiratory quotients. This is the practice followed in much of the recent work in human metabolism and in most of the work reported from the nutrition laboratory of the Carnegie Institution of Washington, and defended on the basis of the small errors incurred (4).

The respiratory quotients obtained during the two fasting experiments, representing in each case observations obtained during the

fourth, fifth, and sixth days of fast, with no preliminary preparation of the steer, are given in Table 14. In the 4-hour samples of chamber air, respiratory quotients below that of fat were frequently obtained, and even the average gas analyses for the entire day indicated quotients slightly below 0.7 in half the cases. In these cases the heat production was computed on the assumption of 100 per cent fat metabolism and a heat equivalent of the consumed oxygen of 4.686 Calories per liter.

TABLE 14.—*Respiratory quotients obtained during two fasting experiments*

Experiment No.	Respiratory quotient in indicated 4-hour periods						Respiratory quotient for 24-hour period
	8 a. m. to 12 m.	12 m. to 4 p. m.	4 p. m. to 8 p. m.	8 p. m. to 12 p. m.	12 p. m. to 4 a. m.	4 a. m. to 8 a. m.	
16.....	0.71	0.74	0.66			0.63	0.70
17.....	.66	.73	.70	0.73	0.72	.75	.71
18.....	.72			.68	.67	.66	.71
25.....	.68	.68	.70	.69	.71	.70	.69
26.....	.68	.70	.67	.69	.68	.67	.68
27.....	.70	.68	.69	.69	.65	.69	.68

Before studying the final computations of the experiment, assembled in Table 17, it is advisable to consider the results of the fasting experiments to be found in Table 15. These computations of heat production did not involve any corrections for methane, since the methane production of the steer after the third day of fasting is inconsiderable (9, 10).

TABLE 15.—*Heat production of the steer during the fourth, fifth, and sixth days of fast*

Body weight of steer	Body surface of steer	Date	Day of fast	Heat production			
				Observed	Per standard day		
					Total	Per square meter	Per 500 kilograms
Kilograms	Square meters			Calories	Calories	Calories	Calories
628.0	5.33	April 16-17.....	4	9,842	9,855	1,850	8,674
		April 17-18.....	5	9,866	9,590	1,800	8,441
		April 18-19.....	6	9,285	9,499	1,780	8,352
		June 4-5.....	4	9,532	9,499	1,914	8,550
602.8	4.96	June 5-6.....	5	9,514	9,480	1,911	8,537
		June 6-7.....	6	9,476	9,399	1,895	8,464

In correcting the observed heat production to a standard day of 12 hours each in the standing and lying positions, following the practice initiated by Armsby and his associates, a factor of 62 Calories per hour per 502 kg of body weight was taken as the extra heat production of standing over lying. This factor is the average of two values obtained during the preceding year on the same steer at a body weight of 500 kg and on another Shorthorn steer at a body weight of 447 kg, on the ninth and tenth days of fast, respectively. During these days

the hourly production of carbon dioxide was obtained for considerable periods of time in the standing and in the lying position. For the first steer the hourly CO₂ production while lying (4 periods of observation totaling 5.17 hours) was 81.07 g, and that while standing (4 periods totaling 11.79 hours) was 98.24 g, an increase of 21 per cent. In this experiment the increment in heat production due to standing amounted to 60 Calories per hour per 500 kg body weight. For the other steer the hourly production of CO₂ while lying (6 periods totaling 3.67 hours) was 81.75 g, and while standing (3 periods totaling 7.45 hours) 98.16 g, an increase of 20 per cent, or, in terms of heat production, 64 Calories per hour per 500 kg.

The surface area of the steer was determined at each of the fasts by the use of the Brody surface integrator (7). In the first fasting experiment the area ⁴ was 5.33 m², and in the second 4.96 m². These values are somewhat larger than those computed from Brody's formula for beef cattle $A = 0.13 W^{.56}$ i. e., 4.80 and 4.69 m², respectively.

The fasting heat production (corrected to the standard day) per square meter of body surface averaged 1,810 Calories for the first fasting period and 1,907 Calories for the second fasting period, or, using the surface areas as determined by the Brody formula, 2,010 and 2,017 Calories, respectively.

In the respiration experiments of the preceding year, already referred to, short observations of 1.5 to 2.0 hours were made on the carbon dioxide production of four grade Shorthorn steers, including the one used in the present experiments, on the fourth or the fifth day of absolute fast. The heat production was calculated on the assumption of a fasting respiratory quotient of 0.72. The results of these experiments are given in Table 16, and relate to the standing position.

TABLE 16.—*The heat production of fasting steers on fourth and fifth days of the fast*

Steer No.	Body weight	Surface area ^a	Day of fast	CO ₂ per hour	Heat production per 24 hours		
					Observed	Corrected to standard day ^b	
						Total	Per square meter of body surface
	Kilograms	Square meters		Grams	Calories	Calories	Calories
1	501.7	4.23	Fourth.....	106.7	8,507	7,761	1,835
2	447.2	3.96	do.....	100.6	8,021	7,356	1,858
3	433.6	3.90	Fifth.....	100.9	8,045	7,508	1,925
4	402.8	3.74	do.....	87.8	6,960	6,461	1,727

^a Computed by the Brody formula, $A = 0.13 W^{.56}$.

^b Using the factor 62 Calories per hour per 500 kg of body weight for the excess heat produced in the standing position.

The values for the heat production per standard day per square meter of surface area are quite similar to those obtained in the later experiment on steer 1. They are, however, considerably greater than

⁴ m² is the abbreviation for square meter recently adopted by the Style Manual for United States Government printing.

those given for fasting steers and dairy cows, by Forbes, Fries, Braman, and Kriss (11) and by Forbes, Kriss, and Braman (12), averaging approximately 1,400 Calories. In two recent publications from the Pennsylvania laboratory (9, p. 276; 10, p. 51) the fasting metabolism of the four steers used as subjects was 1,588, 1,628, 1,642, and 1,491 Calories per square meter of surface area. However, the former values were obtained by the use of Moulton's formula for the surface area of beef cattle (18), based upon the measured area of the removed hides of a number of animals, while the latter four values were based upon the area of the hides of the experimental animals themselves, removed and measured after slaughter. This method of determining surface area may be expected to give larger areas than those obtained by the Brody surface integrator from the live animals. The Moulton formula applied to the steer in the experiment under discussion gives areas of 6.22 and 6.07 m² for the two fasting periods, and heat productions per standard day per square meter of 1,550 and 1,558 Calories. It may be concluded, therefore, that the fasting heat productions of steers in the Illinois experiments are quite consistent with those obtained at the Pennsylvania laboratory when computed to the unit of surface area by the same formula. The average of 13 determinations of the fasting heat production of beef cattle, corrected to the standard day, from the Illinois and Pennsylvania experiments, is $1,897 \pm 19$ Calories per square meter of body surface, the latter computed according to the Brody formula. The standard deviation of these 13 determinations was 100 and their coefficient of variation 5.27.

The experiments of Benedict and Ritzman (6) on four fasting steers gave average values for the heat production per day (in the standing position) per square meter of surface of 1,474, 1,591, 1,590, and 1,570 Calories on the fourth and fifth (or the fourth or fifth) days of fast. These investigators have obtained such discordant values for the influence of standing on the heat production that they have not attempted a correction to the standard day. The surface areas of the steers were estimated in these experiments by the Hogan modification of the Moulton formula (13), namely, $A = 0.1081 W^{5/8}$.

It should be noted that the values given in Tables 15 and 16 were obtained with no attempt to standardize the ration consumed previous to the fast and with no attempt to empty the intestinal tract of the steer by the use of cathartics, procedures used in the Pennsylvania experiments. The fasting heat production in the second experiment (Table 15), secured after a period of feeding at the one-fifth full feed level was, in fact, slightly greater per square meter of surface than the fasting heat production in the first experiment, secured after a period of feeding at the three-fifths full feed level. In the earlier series of experiments (Table 16) the steers were, previous to the fast, consuming a fattening ration of alfalfa hay and corn upon which they had been making gains of 2.49, 1.79, 1.36, and 1.54 pounds daily, respectively, for a period of 44 days. There appears to be no indication that the level of feeding previous to the fast has any appreciable influence on the heat production of fasting after the third day. This does not seem a matter of surprise to the writers, since, even though the paunch may still contain considerable food, its passage from the paunch into the third and fourth stomachs and

the intestinal tract would presumably be very slow, as would also be the rate of its digestion and absorption. Experiments referred to at the beginning of this paper, as well as the experiment now under discussion indicate clearly that the digestion and absorption of small amounts of food have only an inappreciable effect upon heat production.

In Table 15 will be found computations of the fasting heat production of the steer per 500 kg body weight, computed in the proportion of the 0.56 power of the weight. The six computations agree fairly well and average 8,503 Calories. This figure has been used in the computations of the fasting heat production of the steer during the five feeding periods (Table 17), in estimating the heat increment due to feeding. The surface area of the steer was not directly determined during these periods.

TABLE 17.—*Calculation of the heat increments and the net energy values of the rations*

Plane of nutrition	Dry matter consumed	Metabolizable energy intake	Observed heat production	Energy balance	Estimated heat production of fasting: Standard day	Activity increment	Total heat production of fasting	Heat increment due to feed	Heat increment per kilogram of dry matter consumed	Net energy		
										Total	Per kilogram of dry matter consumed	In percentage of metabolizable energy
	<i>Kilograms</i>	<i>Calo-ries</i>	<i>Calo-ries</i>	<i>Calo-ries</i>	<i>Calo-ries</i>	<i>Calo-ries</i>	<i>Calo-ries</i>	<i>Calo-ries</i>	<i>Calo-ries</i>	<i>Calo-ries</i>	<i>Calo-ries</i>	<i>Per cent</i>
Full feed.....	11.229	29,881	19,170	10,711	9,604	+180	9,784	9,386	836	20,495	1,825	68.6
	11.229	29,881	18,654	11,227	9,639	+10	9,649	9,005	802	20,870	1,859	69.9
Average.....	11.229	29,881	18,912	10,969	9,621	-----	9,717	9,195	819	20,685	1,842	69.3
Four-fifths.....	8.975	24,320	16,255	8,065	9,342	+334	9,676	6,579	733	17,741	1,977	72.9
	8.975	24,320	15,486	8,834	9,294	+90	9,384	6,132	689	18,188	2,027	74.8
	8.975	24,320	10,025	8,295	9,394	+257	9,651	6,374	710	17,940	2,000	73.8
Average.....	8.975	24,320	15,922	8,398	9,343	-----	9,560	6,361	709	17,958	2,001	73.8
Three-fifths.....	6.806	18,982	14,901	4,081	9,631	+376	10,007	4,894	719	14,088	2,070	74.2
	6.806	18,982	13,600	5,382	9,702	-293	9,409	4,191	616	14,791	2,173	77.9
	6.806	18,982	14,760	4,222	9,655	+68	9,723	5,037	740	13,945	2,049	73.5
Average.....	6.806	18,982	14,420	4,562	9,663	-----	9,713	4,707	692	14,275	2,097	75.2
Two fifths.....	4.644	13,563	12,387	1,176	9,545	-47	9,498	2,889	622	10,674	2,298	78.7
	4.644	13,563	12,559	1,004	9,496	-49	9,447	3,112	670	10,451	2,250	77.1
	4.644	13,563	12,610	953	9,549	-2	9,547	3,063	660	10,500	2,261	77.4
Average.....	4.644	13,563	12,519	1,044	9,530	-----	9,497	3,021	651	10,542	2,270	77.7
One-fifth.....	2.323	7,187	10,047	-2,860	9,436	+119	9,555	492	212	6,695	2,882	93.2
	2.323	7,187	9,548	-2,361	9,436	-67	9,369	179	77	7,008	3,017	97.5
	2.323	7,187	9,670	-2,483	9,436	-48	9,388	282	121	6,905	2,972	96.1
Average.....	2.323	7,187	9,755	-2,568	9,436	-----	9,437	318	137	6,869	2,957	95.6

The final results of the experiments are contained in Table 17. In the fourth column of this table will be found the observed heat production of the steer for 24 hours, computed as above explained. The difference between these values and the daily intakes of metabolizable energy yields the energy balances, which averaged for the different periods, in the order of decreasing food consumption, 10,969, 8,398, 4,562, 1,044, and -2,568 Calories. It appears from these

figures that the level of feeding equal to two-fifths full feed was slightly above the maintenance level. To obtain the heat increments, the fasting heat production (standard day) plus the estimated heat liberated due to standing for more than 12 hours must be deducted from the observed heat production; these values are given in columns 6, 7, and 8. The total heat increment and the increment computed to the kilogram of dry matter will be found in columns 9 and 10. The latter values for the five levels of feed are, in the order of decreasing food consumption, 819, 709, 692, 651, and 137 Calories. The net energy results are given in the last three columns. Per kilogram of dry matter consumed, the net energy values averaged, in the same order of feeding, 1,842, 2,001, 2,097, 2,270, and 2,957 Calories. The percentage availability of the metabolizable energy averaged 69.3 at the level of full feed, 73.8 at four-fifths full feed, 75.2 at three-fifths full feed, 77.7 at two-fifths full feed, and 95.6 at one-fifth full feed.

The biological error in this experiment is represented by the differences between the daily heat productions at each level of feeding. The average percentage difference between each two days' results at each of the five levels of feeding and of the two fasting periods was 3.02. If these observed heat productions are corrected to a standard day, by means of the activity increments given in column 7 of Table 17, the average difference becomes 2.29 per cent (standard deviation, 1.93), and in all but 5 of the 19 comparisons the correction for activity resulted in smaller differences. In two recent publications by Forbes and his associates (9, 10), the average difference in observed daily heat productions on the same level of feeding, uncorrected to a standard day, was 1.92 per cent, standard deviation, 1.43. In the compilation of early experiments from the same laboratory in 1915, published by Armsby and Fries (2), the average percentage difference between duplicate determinations of daily heat production corrected to the standard day is 2.73, the standard deviation of the 75 differences being 2.19. In the consideration of the necessary degree of refinement in the technic of animal calorimetric investigations the extent of this inevitable biological error must find a place. It is of particular importance in assessing the significance of small differences in energy metabolism caused by differences in the plane of nutrition.

DISCUSSION OF RESULTS

The direct experimental measurement of the heat increment due to food is the difference between the observed heat production and the fasting heat production, corrected to equal body weight and, in so far as possible, to equal muscular activity. The use of lower levels of feeding in the computation of heat increments in place of the fasting condition gives values of uncertain significance. Their interpretation would seem to involve necessarily some particular theory concerning the factors responsible for the heating effect of food. For example, if the heat production on a maintenance ration is used in the computation of a heat increment between it and some higher level of feeding, the implication would seem to be that the excess feed above maintenance has itself suffered a loss in energy equal to this computed increment. It has been found that this increment per kilogram of dry matter consumed is greater than the

increment obtained between fasting and maintenance. The conclusion has therefore been drawn that the utilization of feed energy in fattening is less than that of maintenance. But this implies that the heating effect of food varies with the purpose for which it is used, and this follows only from one of a number of possible theories of the cause of this effect, namely, that it is due to heat liberated in the course of the chemical reactions of intermediary metabolism (16).

If the heat increment computed between maintenance and some higher level of feeding is not related only to the excess food, but also represents an increase in the heat loss suffered by the maintenance ration itself, then the increment possesses a mixed significance and can not logically be used, for example, in computing the net energy value of the ration for fattening only. In this case the heat increment might be the result entirely of the level of feeding and of the plethora of food nutrients set up in the blood and the intercellular fluids. The percentage utilization of the metabolizable energy would then suffer a decrease with increasing levels of feeding quite analogous to the concomitant decrease in the percentage digestibility of the food nutrients. Hence, it would be as illogical to compute a separate value for the net energy content of food consumed above maintenance as to compute separately its digestibility.

Until the causes underlying the heating effect of food, including its specific dynamic action, are much more completely understood than they are at present, it would seem to be the preferable procedure to interpret the results of this experiment, and of others similar to it, in the most direct fashion possible, giving to the total heat increments and the corresponding net energy values their simplest interpretations.

In the discussion which follows, the results of this experiment on one steer will be compared with the results, obtained in similar experiments on four steers by Forbes and his associates (9, 10). These are the only published experiments, in so far as the writers are aware, that have been concerned with the effect of the level of nutrition upon the energy metabolism of steers and upon their utilization of food energy.

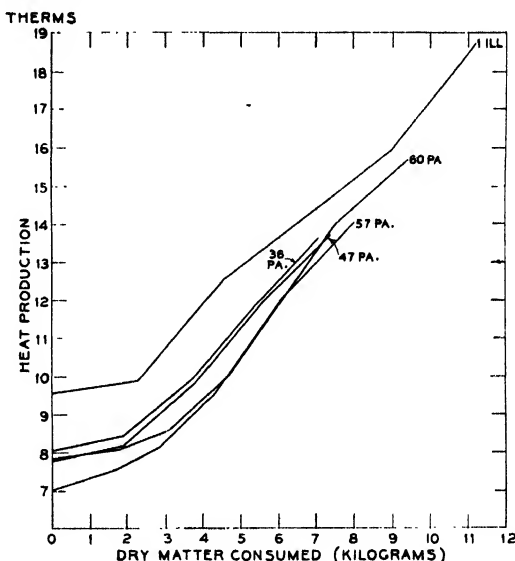


FIGURE 3.—Relation between heat production of steers and the quantity of dry matter consumed daily, illustrated by data from this experiment and from experiments of Forbes and his associates

In Figure 3 the relation between the heat production of the five steers, corrected to a constant body weight, and the dry matter consumed daily, is depicted graphically. The broken lines representing this relation are quite similar in their general trend. From the fasting condition to the lowest level of feeding, the heat production increases but slightly. From the lowest to the next feed level, representing for all steers approximately a maintenance ration, the heat production increases more rapidly. From these points to higher levels the heat production increases in an approximately linear fashion, the slight irregularities being apparently of no significance since they are not consistent. The intervals between the five lines are largely due to the differences in the body weights of the steers to which the heat productions have been corrected. The two lowest curves are for the two lightest steers, Nos. 57 Pa. and 60 Pa., with weights of

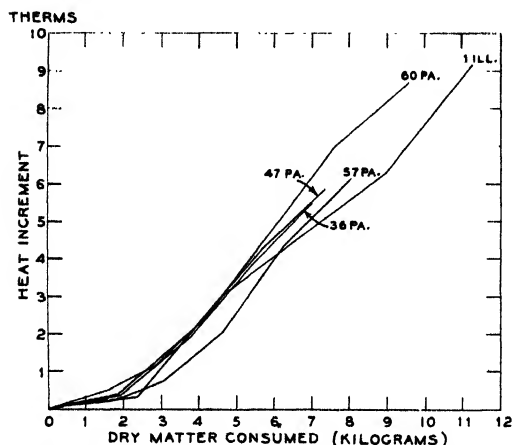


FIGURE 4.—Relation between total heat increment and quantity of dry matter consumed daily, illustrated by data from this experiment and from experiments of Forbes and his associates

399 and 383 kg, respectively. The two intermediate curves are for the next heaviest steers, Nos. 36 Pa. and 47 Pa., weighing 468 and 479 kg, respectively. The heat production of the Illinois steer was corrected to the average weight of the two fasting periods, namely, 615 kg.

The curves showing the relation between the total heat increments and the dry matter consumed (fig. 4) are much closer together except at the higher levels of feeding.

The discrepancy here between steers 60 Pa. and 1 Ill., which were carried to higher levels of feeding than the others, may be due to individuality or possibly to the differences in ration, the ration of the Illinois steer containing more concentrates.

When the net energy values of the rations per kilogram of dry matter are plotted against the daily intake of dry matter, as in Figure 5, it appears that, except for the lowest levels of intake for steers 57 Pa., 60 Pa., and 1 Ill., the relation is more or less perfectly a linear one. The lines drawn upon this chart have been determined from the data of each steer (with the exceptions noted) by the method of least squares. The difference in slope between the line for the Illinois steer and the lines for the Pennsylvania steers may be due to the difference in the character of the rations fed.

The goodness of fit of these lines to the data of the five steers is indicated in Table 18, which contains the observed net energy values of the rations at the different levels of intake and the values estimated from the equations describing the straight lines, given in the last column of the table. For steers 47 Pa. and 36 Pa. it appears to be

possible to predict by linear equations the net energy value of the ration, for all levels of intake observed, with an error of less than 1 per cent. For steers 60 Pa. and 57 Pa., the predictions fall within 5 per cent of the observed values, with the exception of the results of the lowest levels of intake, which do not fall in line with the others and for which no predictions have been made. For the Illinois steer, again neglecting the result of the lowest level of feeding, the net energy value of the ration can be predicted within 1 per cent by the linear equation fitted to the data.

Although it might be supposed that the net energy value of a ration could be predicted considerably more accurately from the intake of metabolizable energy than from the intake of dry matter, this did not prove to be the case with steers 60 Pa. and 57 Pa., for which the dry matter net energy relation deviated the most from linearity. For the prediction on the basis of dry matter intake, the average percentage deviations between observed and predicted values for these two steers were 3.24 and 2.20, respectively. On the basis of the intake of metabolizable energy, the average percentage deviations between the observed net energy values and those predicted from a linear equation were 2.94 and 2.20, respectively. The same situation existed in the case of the other three steers.

It would appear that the problem of determining the net energy value of a ration has become more complicated as the result of the demonstration in a number of laboratories of the influence of the plane of nutrition. If the few results illustrating this influence in steers may be taken as a good indication of the truth, it seems that for each ration the net energy value may be expressed, not as a definite point in accordance with the Armsby system, but better as a linear equation relating the net energy value to the dry matter intake. Thus, from the Pennsylvania results, the net energy value of a ration consisting of equal parts of alfalfa hay and corn can not be assigned a definite value for all conditions of feeding, but may be predicted for all levels of feeding above maintenance from the linear equation $y = 2.633 - 0.1108x$, in which x is the dry matter consumed in kilo-

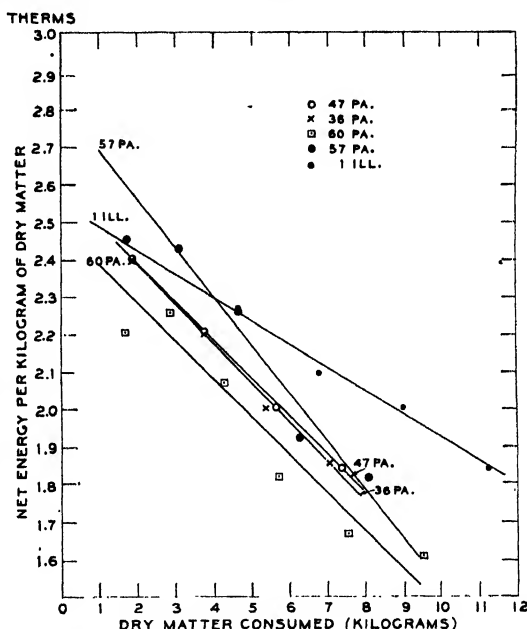


FIGURE 5.—The net energy value of various rations as affected by the quantity of dry matter consumed; illustrated by data from this experiment and from experiments of Forbes and his associates

grams and y is the net energy of the ration per kilogram of dry matter. The constants in this equation are the averages of the constants of the four equations for the individual steers. For low levels of nutrition (submaintenance (?)), the validity of a linear relation has not been established. More investigation needs to be done in clarifying the relation between net energy value and dry matter consumed at these levels.

Wiegner and Ghoneim (21) have described the relation between the intake of metabolizable energy and that of net energy, both expressed per square meter of body surface, by the differential equation

$$\frac{dA}{dF} = K(H - A)$$

in which A is the net energy intake, F the intake of metabolizable energy, H is the maximum value of A , and K is an efficiency constant.

TABLE 18.—*Prediction of net energy value of ration from the quantity of dry matter consumed, using Pennsylvania and Illinois data*

Steer No.	Dry matter consumed daily (x)	Net energy of ration per kilogram of dry matter			Difference	Prediction equation
		Observed	Calculated (y)			
	Kilogram	Therms	Therms	Per cent		
47 Pennsylvania.....	7.384	1.842	1.839	-0.2	} $y = 2.622 - 0.1060x.$	
	5.617	2.012	2.027	+7		
	3.790	2.241	2.220	-1.0		
	1.863	2.414	2.425	+4		
36 Pennsylvania.....	7.037	1.857	1.857	0	} $y = 2.605 - 0.1065x.$	
	5.353	2.027	2.035	+4		
	3.762	2.218	2.204	-7		
	1.885	2.399	2.404	+2		
60 Pennsylvania.....	9.489	1.605	1.528	-4.8	} $y = 2.485 - 0.1009x.$	
	7.520	1.667	1.726	+3.5		
	5.704	1.823	1.909	+4.7		
	4.237	2.071	2.057	-7		
57 Pennsylvania.....	2.828	2.257	2.290	-2.5	} $y = 2.821 - 0.1299x.$	
	8.057	1.818	1.774	-2.3		
	6.233	1.923	2.011	+4.6		
	4.612	2.260	2.222	-1.7		
1 Illinois.....	3.085	2.426	2.420	-2	} $y = 2.538 - 0.0615x.$	
	11.229	1.842	1.847	+3		
	8.975	2.001	1.986	-7		
	6.806	2.097	2.119	+1.0		
	4.644	2.266	2.252	-6		

The integrated form of this equation, $1n \frac{H}{H-A} = KF$, was fitted to their own data on a rabbit and to the Pennsylvania data on four steers. In the latter case the average percentage deviation between observed and computed data was 3.9 and the maximum 11.7, one equation being used, i. e.,

$$1n \frac{5011}{5011-A} = 0.0001995F$$

One may question the significance of reducing total intakes of energy to the square meter of body surface.

SUMMARY AND CONCLUSIONS

The energy metabolism of a grade Shorthorn steer, approximately 2 years of age, has been investigated at six levels of nutrition, ranging from fasting to full feed. The ration used was the same at all levels, containing on the dry matter basis 73.1 per cent of ground corn, 24.1 per cent of alfalfa hay, 2.0 per cent of linseed meal, and 0.8 per cent of molasses.

The lowest level of feeding was associated with the most complete digestibility of all nutrients. However, there was a progressive decrease in digestibility from the lowest to the highest ration only in the case of nitrogen-free extract, ether extract, and dry substance.

The metabolizable energy of the ration per kilogram of dry matter, as well as the percentage metabolizability of the gross energy, increased progressively from the highest to the lowest level of feeding, the former from 2,661 Calories to 3,094 Calories, and the latter from 60.61 per cent to 70.14 per cent. However, when the metabolizable energy is computed to a kilogram of digestible nutrients, all effect of the level of feeding disappears.

At the two highest levels of feeding, the respiratory quotient was continuously greater than 1 for the entire day. At all levels of feeding except the lowest, the respiratory quotient for the first four hours after feeding was higher than for any other period. The average daily respiratory quotients for the five levels of feeding in the order of decreasing levels were 1.13, 1.12, 1.05, 0.97, and 0.88.

The average fasting heat production of the steer, corrected to a standard day of 12 hours in the standing position and 12 hours in the lying position, averaged 1,858 Calories per square meter of body surface, as directly determined with the Brody surface integrator. If the surface area of the steer is computed by the Moulton formula, based upon skin areas, a much larger value is obtained than that actually observed, and the fasting heat production per square meter becomes 1,554 Calories per day, a value consistent with previously published values for steers. Serious discrepancies exist between formulas now being used for the estimation of the surface area of steers.

The average of 13 determinations of the fasting heat production of beef cattle, corrected to the standard day, from the Illinois and Pennsylvania experiments, is $1,897 \pm 19$ Calories per square meter of body surface, the latter computed according to the Brody formula. The standard deviation of these 13 determinations was 100 and their coefficient of variation 5.27.

In this experiment, although one fasting period followed a three-fifths full-feed period and one a one-fifth full-feed period, no evidence was obtained that the preceding feeding affects appreciably the heat production of the fourth, fifth, and sixth days of fast. Other experiments performed in this laboratory are cited to the same effect.

The heat increment due to feeding, per kilogram of dry matter consumed, increased from the lowest to the highest levels of feeding, while the net energy values, on the same basis, decreased progressively from 2,957 Calories to 1,842 Calories. The percentage availability of the metabolizable energy assumed the following values from the lowest to the highest levels of feeding: 95.6, 77.7, 75.2, 73.8, and 69.3. This decrease in total utilization of feed by steers seems to be due in

part to a more or less continuous decrease in digestibility, but also to a very slight specific dynamic effect of feed at very low levels of feeding.

From the results of this experiment and of the experiments on the same problem published by Forbes and his associates, it appears that, except for submaintenance levels of feeding, the net energy value of a ration bears a linear relation to the amount of dry matter consumed. Hence, the net energy value of a ration may be defined, not by a constant, as Armsby supposed, but by a linear equation relating it to the intake of dry matter. There are grounds for suspecting that the size of steer may eventually have to be considered also in defining this relation, but at present the data are not at hand to decide this question.

LITERATURE CITED

- (1) ANDERSEN, A. C.
1922. ZUR AUSFÜHRUNG UND BERECHNUNG VON STOFFWECHSELVERSUCHEN MIT WIEDERKÄUERN. *Biochem. Ztschr.* 130 : 143-150.
- (2) ARMSBY, H. P., and FRIES, J. A.
1915. NET ENERGY VALUES OF FEEDING STUFFS FOR CATTLE. *Jour. Agr. Research* 3 : 435-491, illus.
- (3) BENEDICT, C. G., and BENEDICT, F. G.
1923. A PERMISSIBLE BREAKFAST PRIOR TO BASAL METABOLISM MEASUREMENTS. *Boston Med. and Surg. Jour.* 188 : 849-851.
- (4) BENEDICT, F. G., and CARPENTER, T. M.
1918. FOOD INGESTION AND ENERGY TRANSFORMATIONS, WITH SPECIAL REFERENCE TO THE STIMULATING EFFECT OF NUTRIENTS. 355 p., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 261.)
- (5) ——— and JOSLIN, E. P.
1910. METABOLISM IN DIABETES MELLITUS. 234 p., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 136.)
- (6) ——— and RITZMAN, E. G.
1927. THE METABOLISM OF THE FASTING STEER. 246 p., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 377.)
- (7) BRODY, S., and ELTING, E. C.
1926. GROWTH AND DEVELOPMENT WITH SPECIAL REFERENCE TO DOMESTIC ANIMALS. II. A NEW METHOD FOR MEASURING THE SURFACE AREA AND ITS UTILIZATION TO DETERMINE THE RELATION BETWEEN GROWTH IN SURFACE AREA AND GROWTH IN WEIGHT AND SKELETAL GROWTH IN DAIRY CATTLE. *Missouri Agr. Expt. Sta. Research Bul.* 89, 18 p., illus.
- (8) CARPENTER, T. M., FOX, E. L., and SEREQUE, A. F.
1929. THE CARPENTER FORM OF THE HALDANS GAS ANALYSIS APPARATUS. CHANGES MADE IN THE APPARATUS AND DETAILS REGARDING ITS USE. *Jour. Biol. Chem.* 83 : 211-230, illus.
- (9) FORBES, E. B., BRAMAN, W. W., and KRISS, M., with the collaboration of JEFFRIES, C. D., SWIFT, R. W., FRENCH, R. B., MILLER, R. C., and SMYTHE, C. V.
1928. THE ENERGY METABOLISM OF CATTLE IN RELATION TO THE PLANE OF NUTRITION. *Jour. Agr. Research* 37 : 253-300, illus.
- (10) ——— BRAMAN, W. W., and KRISS, M., with the collaboration of SWIFT, R. W., FRENCH, R. B., SMYTHE, C. V., WILLIAMS, P. S., and WILLIAMS, H. H.
1930. FURTHER STUDIES OF THE ENERGY METABOLISM OF CATTLE IN RELATION TO THE PLANE OF NUTRITION. *Jour. Agr. Research* 40 : 37-78, illus.
- (11) ——— FRIES, J. A., BRAMAN, W. W., and KRISS, M.
1926. THE FASTING KATABOLISM OF DRY COWS. *Jour. Agr. Research* 33 : 591-595.
- (12) ——— KRISS, M., and BRAMAN, W. W.
1927. THE COMPUTED AS COMPARED WITH THE DIRECTLY OBSERVED FASTING KATABOLISM OF CATTLE AS A MEASURE OF THE MAINTENANCE REQUIREMENT OF ENERGY. *Jour. Agr. Research* 34 : 167-179.

- (13) HOGAN, A. G., and SKOUBY, C. I.
1923. DETERMINATION OF THE SURFACE AREA OF CATTLE AND SWINE. *Jour. Agr. Research* 25: 419-430, illus.
- (14) LUSK, G.
1928. THE ELEMENTS OF THE SCIENCE OF NUTRITION. Ed. 4, reset, 844 p., illus. Philadelphia and London.
- (15) MAGNUS-LEVY, A.
1907. THE PHYSIOLOGY OF METABOLISM. Anglo-Amer. issue under the editorship of I. W. Hall. 452 p. Chicago. In Noorden, C. von, *Metabolism and Practical Medicine*, v. 1.
- (16) MITCHELL, H. H.
1927. DOES THE NET ENERGY VALUE OF FOOD DEPEND UPON THE PURPOSE FOR WHICH IT IS USED IN THE BODY? *Science* (n. s.) 66: 289-292.
- (17) MØLLGAARD, H.
1929. FÜTTERUNGSLEHRE DES MILCHVIEHS. DIE QUANTITATIVE STOFFWECHSELMESSUNG UND IHRER BISHERIGEN RESULTATE BEIM MILCHVIEH. 246 p., illus. Hannover.
- (18) MOULTON, C. R.
1916. UNITS OF REFERENCE FOR BASAL METABOLISM AND THEIR INTERRELATIONS. *Jour. Biol. Chem.* 24: 299-320, illus.
- (19) RITZMAN, E. G., and BENEDICT, F. G.
1929. SIMPLIFIED TECHNIQUE AND APPARATUS FOR MEASURING ENERGY REQUIREMENTS OF CATTLE. *N. H. Agr. Expt. Sta. Bul.* 240, 30 p., illus.
- (20) SODERSTROM, G. F., BARR, D. P., and DU BOIS, E. F.
1918. CLINICAL CALORIMETRY. TWENTY-SIXTH PAPER. THE EFFECT OF A SMALL BREAKFAST ON HEAT PRODUCTION. *Arch. Int. Med.* 21: 613-620, illus.
- (21) WIEGNER, G., and GHONEIM, A.
1930. ÜBER DIE FORMULIERUNG DER FUTTERWIRKUNG. EIN BEITRAG ZUR THEORIE DER VERWERTUNG DES UNTERERNÄHRUNGS- UND PRODUKTIONSFUTTERS AUF GRUND VON NEUEN FÜTTERUNGSVERSUCHEN. *Tierernährung* 2: 193-232, illus.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 45

WASHINGTON, D. C., AUGUST 15, 1932

No. 4

STURMIA INCONSPICUA MEIGEN, A TACHINID PARASITE OF THE GIPSY MOTH¹

By R. T. WEBBER²

Associate Entomologist, Division of Forest Insects, Bureau of Entomology, United States Department of Agriculture

INTRODUCTION

Sturmia inconspicua Meigen is recognized as one of the most important parasites of the introduced pine sawfly, *Diprion simile* (Hartig) (*Lophyrus similis* Htg.). As a parasite of the gipsy moth (*Porthetria dispar* L.) it is not so well known, although its attack upon that species was recorded many years ago. As a parasite of *P. dispar* in Europe it compares favorably with other tachinids except *Phorocera agilis* Robineau-Desvoidy and *Sturmia scutellata* Robineau-Desvoidy. In certain localities it has even outranked the latter parasite. Illustrative of the periodic abundance of this species are the rearings made at Rembertow, Poland, in 1927. Approximately 200,000 *P. dispar* larvae were reared for their parasites, and from them were obtained more than 20,000 puparia of *S. inconspicua*.

This increased knowledge of the insect's importance as a gipsy-moth parasite and the establishment in the northeastern part of the United States of one of its favorite hosts, *Diprion simile*, have renewed interest in this species.

SYNONYMY

European authorities do not wholly agree upon the taxonomic status of *Sturmia inconspicua*. According to Bezzi and Stein,³ *inconspicua* Meig. (1830), *flavoscutellata* Zett. (1844), and *cursitans* Rond. (1861) are synonyms of *bimaculata* Htg. (1838). It is not clear why these authors used *bimaculata* when *inconspicua* has priority. Probably it is an instance of common usage. *S. gilva* Htg. (1838) is regarded by them as distinct. In a later work Stein⁴ still holds to his opinion as regards the distinctiveness of the two forms *bimaculata* and *gilva*, but here he recognizes the priority of *inconspicua* over *bimaculata*. Baer,⁵ like Bezzi and Stein,³ uses *bimaculata* in preference to *inconspicua*, but he considers *gilva* a synonym, or at most a variety, of *bimaculata*. In 1926 J. Villeneuve discussed the synonymy of these forms with the writer. According to him, they are all synonymous with *S. inconspicua*, *gilva* being at most a poorly marked variety.

¹ Received for publication Feb. 1, 1932, issued September, 1932.

² In the preparation of this paper the author was materially assisted by the use of data obtained by C. F. W. Muesebeck and his associates, P. B. Dowden and R. C. Brown, of the Budapest laboratory, and by data obtained from the files at the gipsy moth laboratory, Melrose Highlands, Mass. The writer appreciates the assistance given him by C. W. Collins, T. H. Jones, and W. F. Sellers, of the gipsy moth laboratory, and also the kindness of W. R. Thompson, Farnham House laboratory, London, for his criticisms.

³ BEZZI, M., and STEIN, F. KATALOG DER PALLARTISCHEN DIPTEREN. Bd. 3, p. 227. Budapest. 1907.

⁴ STEIN, F. DIE VERBREITESTEN TACHINIDEN MITTELEUROPA'S NACH IHREN GATTUNGEN UND ARTEN. Arch. Naturgesch. (A) 90 (6): 57. 1924.

⁵ BAER, W. DIE TACHINEN ALS SCHMAROTZER DER SCHÄDLICHEN INSEKTEN. Ztschr. Angerw. Entf. 6: 242. 1920.

GEOGRAPHICAL DISTRIBUTION

Sturmia inconspicua is common to central Europe and northern Africa. According to Fisk,⁶ who refers to it under the name *Zygobothria gilva*, it is more abundant in the Mediterranean countries than in the north. In recent years, however, practically all the material collected has come from northern Europe, especially the area where pine is grown. It is probable that its regional abundance has some connection with the presence of its alternate hosts.

HISTORY OF IMPORTATION AND COLONIZATION

In 1906 the first attempt was made by the State of Massachusetts and the Federal Government to introduce the species into the United States. The colony was small and there is some doubt concerning the point of liberation. Presumably it was in the vicinity of North Saugus, Mass. During 1907-08 but few puparia of this species were received from abroad and no colonies were liberated. In 1909 more than 7,000 puparia were received from France and colonized at Wellesley, Wenham, Stoneham, Byfield, and Melrose Highlands, Mass. In addition 385 puparia were obtained from importations of *Porthetria dispar* from Belgium, Austria, and Germany, and flies were liberated in the same localities. During the following years all attempts to recover the species failed, and it is safe to conclude that the establishment of *inconspicua* from this introduction was unsuccessful.

In recent years another effort has been made to establish the species. During 1923-24 only a small quantity of material was received and no colonization attempted. In 1925, 1,886 puparia were imported from Poland, Hungary, Portugal, and Czechoslovakia. Of this material two colonies, aggregating 1,200 adults, were liberated under unsatisfactory conditions. Less than 60 puparia were received in 1926 and none of the flies was liberated. In 1927 importations totaling 15,607 puparia were received from Poland, Hungary, and Yugoslavia, and approximately 4,798 adults were liberated in Massachusetts, New Hampshire, and Rhode Island. In 1928 nearly 55,000 puparia were received from the same localities, and four colonies, aggregating 28,000 adults, were placed in Massachusetts, New Hampshire, and Rhode Island.

The first recovery of *Sturmia inconspicua* was made during July, 1929, when seven puparia were obtained from *Porthetria dispar* larvae collected at the colony site in Saugus, Mass. This colony was liberated in July, 1927.

DESCRIPTION OF STAGES

The description that follows has been drawn from a large number of specimens of both sexes reared from *Neodiprion dyari* Roh. and *Porthetria dispar* L. at the gipsy moth laboratory. The parent stock was bred from *Lophyrus pini* L. collected at Wlodek, Poland.

⁶ FISKE, W. F. PARASITES OF THE GIPSY AND BROWN-TAIL MOTHS INTRODUCED INTO MASSACHUSETTS. WHERE THEY CAME FROM. WHAT THEY ARE DOING. A GENERAL SURVEY OF THE WORK. p. 39, illus. Boston. 1910.

THE ADULT

Head (fig. 1): Horizontal axis, measured at base of antennae, but little wider than at vibrissae. Front variable; average specimen at widest part measuring, in the male, a little less than and, in the female, a little more than one-third the head width. Eyes bare, bucca from one-eighth to one-fifth the eye height. Front and parafacials usually gray pollinose, the former often with a decided golden tinge that sometimes spreads over the entire face (more pronounced in the females) and bearing, in the male, a row of 10 to 12 and, in the female, from 7 to 9 bristles, the lowest and uppermost ones of which are reclinate, and those of the intermediate region decussate. Outside of and parallel with frontal row, several bristles which in some individuals give the appearance of a double row. These bristles are variable and are sometimes entirely absent in the female. In the male the inner vertical bristle large, the outer vestigial; in the female both the vertical bristles well developed, the outer one less strong. Facial ridges bristly on the lowest fourth; palpi yellow. Antennae usually black, reaching lowest fourth of face, third joint in both sexes from two to two and one-half times length of second. Base of third joint and apex of second sometimes red or yellowish, particularly in the female. Arista tapering from base to apex.

Thorax black, moderately to thickly gray pollinose and bearing before the suture 3 acrostichal and 3 dorsocentral bristles; behind the suture either 3 or 4 acrostichal (usually 3) and 4 dorsocentral bristles. Scutellum black at base, the apical half brownish and bearing 3 pairs of strong marginal bristles besides the decussate apical pair. Disk thickly beset with fine depressed hairs, from which arises 1 strong pair of widely separated bristles. Sternopleura usually with 4 bristles, sometimes 3 and a weaker one. Pteropleural bristle small.

Abdomen black, at least the basal half of segments II, III, IV, and a good part of the pleural and ventral regions moderately gray pollinose; segment I and the apices of the following segments shining black. Segment I with no macrochaetae; segment II with a marginal pair; segment III with a marginal row; and segment IV wholly covered except at extreme base. Sides of the second segment sometimes reddish. In the male, underneath segment III there is a small dense patch of depressed hairs similar to that found in *Zenillia eudryae* Town. and in several species belonging to the genus *Sturmia*. This character is absent in the female. Mid tibiae with 1 bristle on the outer front side near the middle, 1 on the forward side which often appears as an inner bristle, and 2 hind bristles. Hind tibiae thickly ciliate, with 1 longer bristle.

Wings hyaline, third vein usually with one strong bristle at base, sometimes a weaker one present. Apical cell open, ending far from the extreme wing tip.

Genitalia (fig. 2) small, retracted. Inner forceps shining black and tapering to slender points. When viewed from the side they are nearly straight, the basal half densely clothed with fine black hairs which are directed upward; when viewed from behind they are distinctly separated from base to apex, parallel for one-half of their length and then outwardly bowed. Outer forceps light brown, shining, wide at base, and tapering to blunt, rounded points; outer surface sparsely clothed with light-brown hairs. Anterior claspers elongate, slender, brownish, terminating in fine points; posterior claspers short, knoblike, scarcely three times as long as their apical width.

Length, moderate size flies, usually about 7 mm but sometimes as long as 10 mm.

THE EGG

Length from 0.55 to 0.60 mm, width 0.18 to 0.20 mm, depth about equal to width; transparent; moderately elongate-oval in profile, posteriorly rounded and tapering anteriorly; lower side concave. Viewed from above, surface of egg finely punctate, with distinct reticulation.

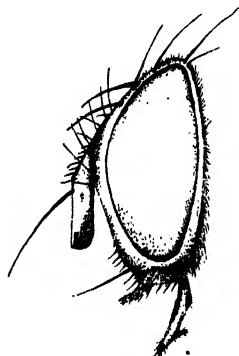


FIGURE 1.—Head of adult female of *Sturmia inconspicua*, lateral view



FIGURE 2.—Male hypopygium of *Sturmia inconspicua*: A, Rear view; B, side view

THE LARVA

Stage 1 (fig. 3, A).—Size variable. A larva 2 days old measured 1.0 mm in length and 0.33 mm in width; another larva, 3 days old, measured but 0.80 by 0.27 mm. Moderately elongate, tapering anteriorly, posteriorly rounded; skin colorless and transparent; head without spines and bearing 2 conspicuous pairs of rodlike sensory organs. On segment I is an anterior band of spines made up of 3 to 5 irregular rows, which are weakest in the pleural region. The spines of the dorsum are less numerous but stronger than those of the venter and stronger than those of any other segment except the anal segment, where the stigmatic spines are located. There is also a much weaker dorsolateral band of 2 or 3 rows commencing near the posterior border of this segment and merging into the anterior row at the pleural region. All spines directed posteriorly. Segment II banded anteriorly with 3 to 5 irregular rows of spines, posteriorly directed, and somewhat weaker than those of the dorso-anterior border of segment I. Rows more numerous in ventral region. Segments III and IV likewise banded anteriorly with 3 to 5 irregular rows of posteriorly directed spines slightly weaker than those of the preceding segment. Posterior two-thirds of segments II, III, and IV apparently naked. Segments V and VI with anterior bands of 3 to 5 irregular rows of posteriorly directed spines which are weakest in the pleural region. Posterior margins of segments sparsely clothed with incomplete rows of minute

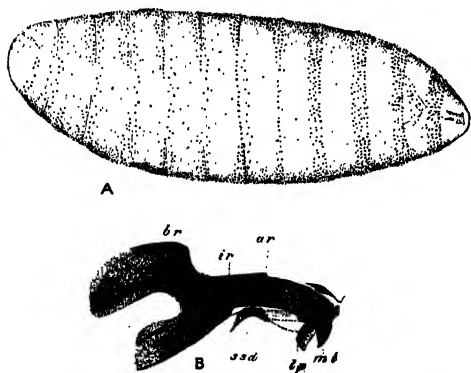


FIGURE 3.—*Sturmia inconspicua*: A, First-stage larva (X 64); B, bucco-pharyngeal armature of same (br, basal region; ir, intermediate region; ar, anterior region; ssd, sclerite of the salivary duct; lp, lateral plates; mt, median tooth)

anteriorly directed spines. Segments VII and VIII with weak anterior bands consisting of from 2 to 4 incomplete rows of posteriorly directed spines which are feeble or missing in the pleural region, also posterior bands of from 2 to 4 rows of anteriorly directed spines which are but narrowly separated from the posteriorly directed spines of the segments which adjoin them. Posterior bands weak along the dorsum. Segments IX and X with incomplete anterior bands of 3 to 5 rows of posteriorly directed spines which are interrupted at the pleural region and strongest at venter. The posterior borders of these segments have more or less complete bands of several rows each of anteriorly directed spines. On segment X the posterior band is broken at venter, but midway to the suture which separates segment IX a short, though well-defined, ventral row of anteriorly directed spines can be observed. Segment XI has no well-marked anterior band of spines, although in some specimens there appears to be a weak, sparsely beset row along the suture that separates segments X and XI. Ventrally, near the middle of segment XI, an irregular row of anteriorly directed spines of varying length, widest at venter and narrowing as they approach the pleural region, where they terminate in a small patch of 5 to 8 strong spines which are of an entirely different character from those which precede them. Region adjacent to posterior spiracles sparsely beset with weak, anteriorly directed spines, inconspicuous in most part, even under high magnification. Anterior to, but directly between, the spiracles there arise 3 to 6 strong, black, anteriorly directed stigmatic spines. Opposite them a somewhat similar posteroventral patch of strong anteriorly directed stigmatic spines. Segment XI further characterized by 2 conspicuous brown sclerotized spots each equal in size to one of the posterior spiracles and situated midway between them and the ventral patch of stigmatal spines. There are also several pairs of papillae on this segment. These papillae are the usual clavate sensorial organs and are about 5 times as long as they are broad.

The first-stage larva is metapneustic, the posterior spiracles opening near the apex of the last segment considerably dorsad of the longitudinal axis. Bucco-

pharyngeal armature without articulations. (Fig. 3, B.) Anterior lateral plates (*lp*) lightly pigmented and irregularly shaped; the sclerite of the salivary duct (*ssd*) large, somewhat shoehorn-shaped with a slender ventral appendage; anterior region (*ar*) with a median tooth (*mt*) directed straight downward, moderately thickened and pointed at tip, bearing on the upper half of its anterior edge a series of 4 or 5 distinct, though minute, accessory teeth. Behind the teeth and arranged in parallel formation are a number of well-defined striae. Basal half of anterior region lightly pigmented. Intermediate region (*ir*) defined on its dorsal anterior edge by a slight hump and on its ventral posterior edge by a marked indentation of the basal region. Upper wing of the basal region (*br*) abruptly curved, at its widest part nearly one and one-half times the width of the intermediate region; lower wing weakly sclerotized at apex and about one-half the size of the upper one.

Stage 2 (fig. 4, A).—Length and width variable. Average measurements 2.0 to 2.5 mm by 0.80 to 1.0 mm. Segment I with dorsal and ventral spinous areas composed, respectively, of 4 and 7 irregular rows of posteriorly directed spines. Pleural region bare. Segments II and III with complete anterior bands composed of 5 to 7 rows of posteriorly directed spines, weakest in pleural region and strongest at venter. Spines of segments I and II stronger than those of any other segments. Posterior borders of segments I and II bare. Segment III with a few weak, scattered spines in the posterior region. Segment IV with a complete anterior band composed of 5 or 6 rows of posteriorly directed spines, also an incomplete ventroposterior band of anteriorly directed spines of the same strength which end in the pleural region. Segment V with an anterior band (sometimes narrowly interrupted at dorsum) of 4 or 5 rows, which are directed posteriorly, and a posterior band of anteriorly directed spines of the same strength, which is also interrupted at dorsum. Segments VI to IX with complete bands of weak spines composed of 5 to 7 rows bordering their anterior and posterior margins. The spines of the ventral region are stronger than those of the dorsum. All spines bordering the anterior edge of segments directed posteriorly, those of posterior edge directed anteriorly. Segment X with weaker and narrower bands of 3 to 5 rows of posteriorly directed spines on its anterior margin. Spine rows of venter more numerous than those of dorsum. Posterior margin of this segment partially banded (interrupted at venter), with numerous irregular rows of anteriorly directed spines much weaker than those of the preceding segments. Segment XI with no well-defined anterior spine bands, but entire dorsum lightly beset with weak spines and the pleural and ventral regions feebly spinose. Posterior region with 5 or 6 irregular rows which vary greatly in strength and arrangement. At venter, between these rows and the suture, a conspicuous ventrolateral patch of weak, anteriorly directed spines. Extreme posterior region bare. Stigmatic spines and sclerites of anal segment, which are characteristic of the first-stage larva, not present in this stage.

Anterior spiracles, if present, inconspicuous and difficult to locate. Posterior spiracles located, as before, on dorsal surface of anal segment and appearing more robust than those of the preceding stage.

The bucco-pharyngeal armature is shown in Figure 4, B. In this stage there is a well-marked joint (*a*), which separates the now paired mandibular hooks from the intermediate section.

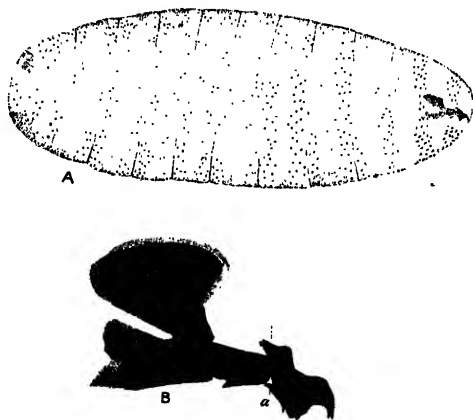


FIGURE 4.—*Sturmia inconspicua*: A, Second-stage larva ($\times 26$); B, bucco-pharyngeal armature of same (*a*, joint separating mandibular hooks from intermediate section)

Stage 3 (fig. 5, A).—Length and width variable. Average measurements 8.0 to 9.0 by 3.0 to 3.5 mm. Head bare except about the mouth, where there is a small patch of posteriorly directed spines. Two pairs of sensory organs conspicuous. Segment I, anterior edge bordered dorsally and ventrally with 6 or 7 irregular rows of short, black, posteriorly directed spines; pleural region weakly spinose (3 or 4 rows); posterior region having few weak spines, in most part outwardly directed. These spines (fig. 5, B) are nearly colorless, unusually long and hairlike, and in decided contrast to those of the anterior border. Segment II, anterior border with 7 or 8 rows of short, black, posteriorly directed spines, strongest in the dorsal and ventral regions; posterior region less thickly clothed with hairlike spines, those nearest the margin, at least, directed anteriorly. Segments III to X with complete anterior bands of 5 or 6 irregular rows of posteriorly directed spines, in most part hairlike, weakest in the pleural regions and strongest ventrally; posterior regions of these segments thickly clothed with the same type of spines, anteriorly directed; intermediate regions less densely spinose. Anal opening situated on posterior margin of segment X, on ventral side. Space

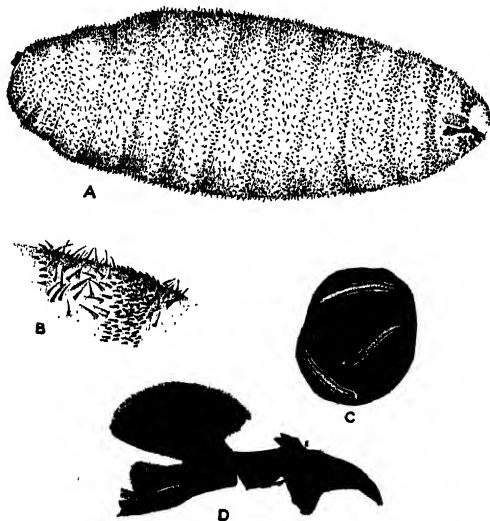


FIGURE 5.—*Sturmia inconspicua*: A, Third-stage larva ($\times 7.5$); B, dorsal view of segment II of same, showing type and arrangement of spines; C, posterior spiracles of same; D, bucco-pharyngeal armature of same

around opening, which is as large as that from which one of the posterior spiracles arises, bare and entirely surrounded by black spines. Segment XI with anterior border clothed with several rows of hairlike spines, in most part posteriorly directed. Posterior extremity, ventrad of the spiracles, defined by a weak suture bordered anteriorly by a dense ventropleural band of anteriorly directed spines; immediate region either side of this band less thickly beset with weak, colorless spines. Except for the bare area adjacent to the spiracles, which is broadest ventrally, and the narrow ventral anterior border of the posterior extremity, which is nearly bare, the segment is thickly beset with anteriorly directed, short, black spines, similar to those which form the anterior bands of segments I and II. Posterior spiracles (fig. 5, C) opaque brown or black, protuberant, their

stalks directed obliquely upward, about as long as broad. Each spiracle with three light brownish respiratory slits, varying somewhat in length and arrangement, located on top of well-defined ridges. In the slide mounts it is difficult, because of their irregular elevation, to obtain a good perspective of the posterior spiracles. Usually they appear as in Figure 5, C, although they are frequently distorted so that only two of the slits are visible. Anterior spiracles, if present, inconspicuous and difficult to locate.

The bucco-pharyngeal armature is represented in Figure 5, D. In this stage there are three articulations which can be readily discerned, although in some cases the segmentation between the intermediate and the anterior regions is not clearly defined. Other differences separating this stage from the preceding one are the elongation of the laterally paired mandibular hooks, the subsequent modification of the sharp angles which characterized the anterior region of the second stage, and the very distinct formation of the intermediate section.

THE PUPARIUM (fig. 6)

Length and width variable, specimens as small as 3 by 1 mm and as large as 7 by 3 mm have been noted. Usually opaque brown or black, but sometimes light

brown and semitransparent, never shining. The long hairs borne on it give a dull appearance and also favor the accumulation of dirt. Posterior spiracles (fig. 6, B and C) located just above the horizontal axis and separated at base by a distance about equal to their width, conspicuously protuberant, the stalk shining black, directed obliquely upward, about as long as broad, each spiracle and three brownish slits each located on top of a well-defined ridge. Button small, located at inner edge of spiracular plate and, in some cases, poorly defined and appearing as a minute shining black dot some distance from the edge. At the base of the tubercles which form the spiracles, below and between them, is a slight elevation with a median depression. Prothoracic spiracles inconspicuous and, if present, extremely difficult to locate on the external surface of the puparium. Anal opening large, located far below the posterior spiracles, near the posterior border of segment X.

SEASONAL HISTORY

In Europe the species passes the winter as a first-stage maggot within the cocooned larva of several species of the old genus *Lophyrus* (sawflies) and in the overwintering larva of *Dendrolimus pini* L. At the beginning of spring the hibernating maggot develops to maturity and, in the case of a sawfly host, escapes from the cocoon through a circular opening cut at one end. The pupal period is about two weeks long. The adult then issues, mates, and in about another two weeks attacks those hosts which are suitable for its perpetuation. The maggot of the next generation develops rapidly within the parasitized host larva, some individuals completing their larval development in six days. The adults of this generation (summer generation) appear about a month later. They behave as did their parents, mating and later ovipositing upon certain host species which are then available. As a rule it is the progeny of this generation that hibernate, although there are apparently some individuals which emerge, thereby forming a partial third generation. At the Budapest laboratory oviposition by the flies of the summer generation was obtained as late as September 12 on *Porthetria dispar* in which development had been retarded for several weeks. Their progeny did not attempt to hibernate, but completed their development, issued, and gave adults between October 20 and 27—that is, after 38 to 45 days. These adults were then mated and later successfully deposited eggs on *Plusia gamma* L. and *Noctua c-nigrum* L. (*Agrotis c-nigrum* L.).

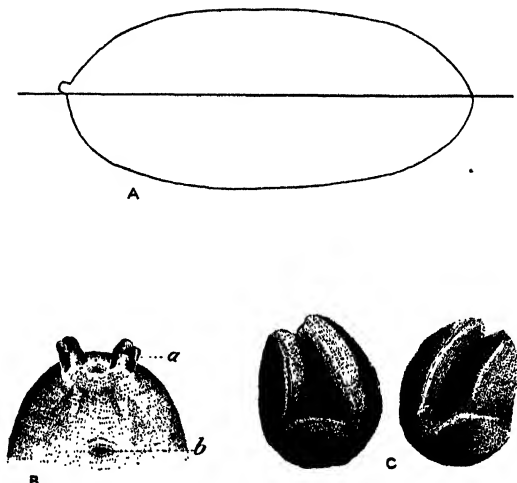


FIGURE 6.—*Sturmia inconspicua*: A, Puparium, lateral view ($\times 8$); B, ventral view of posterior end held at an angle of 45° (a, stigmata; b, anal opening); C, posterior spiracular plates

LIFE HISTORY

MATING

As is usual among the tachinids, the males issue first. Several days elapse between the height of their emergence and that of the females. Mating of the species was readily obtained in cages measuring 14 by 16 by 36 inches. These cages were of wood frame and covered with cloth with the exception of part of the front, which was celluloid. One cage would accommodate 300 flies. In shell vials, 2 by 8 inches, and in smaller containers mating was not so easily obtained.

Usually the females are several days old before mating is effected. In other species instances have been recorded of the males mating with females whose wings were scarcely dried. Of the several thousand flies observed at this laboratory, such an occurrence has not been recorded.

According to observations made at the Budapest laboratory, the mating flies prefer the bright sunlight. It was found that when the flies were moved from the laboratory into the bright out-of-door light mating would take place at once. At the Melrose Highlands, Mass., laboratory, however, the flies apparently mated very well in the semidarkened laboratory.

The pairing of the species is usually preceded by a momentary struggle of the flies; they then settle down and remain more or less stationary for a variable length of time. Ordinarily the flies remain in coitu for several hours, and frequently those taken from the cages during the evening remain so until the following morning. In some cases, however, a pair will separate in less than an hour even though there is no obvious disturbance. Of nine pairs removed from the cages June 8, the time spent in coitu was: $1\frac{1}{4}$ hours for one pair, $4\frac{1}{4}$ hours for four pairs, and more than 5 hours for the remaining pairs.

Males that have mated once will do so a second time and the result of the second union is successful. Females seen in coitu from 15 minutes to 1 hour have been observed to separate and remate after a short time, the second mating continuing for more than 6 hours.

PREOVIPOSITION PERIOD

In 1928 adults began to issue from the first installment of imported material upon its arrival at the laboratory on May 18, and continued issuing until the middle of June.

On May 31 and June 1, 20 pairs of flies (in coitu) were taken from the cages, placed in separate containers, and their behavior noted. Dissection of some females five days after fertilization showed the uterus swollen to many times its original size and packed with minute, elongated maggots each inclosed within a thin membranous eggshell. Occasionally a marked difference in the development of the eggs within the uterus was noted, those at the anterior end being semi-transparent and motionless while those at the posterior end contained fully developed maggots, some of which were active. By actual count there were 97 maggots in the uterus of one female and 102 maggots in that of another.

After the third day of mating, various host larvae were presented to the flies in an attempt to obtain oviposition. This procedure was

continued daily up to the time of oviposition, which usually did not occur until 12 to 14 days after mating. The minimum period for preoviposition was obtained from two females taken in coitu June 8 and separating after two hours. Seven days later these two flies oviposited on the penultimate-stage larvae of the sawfly *Neodiprion dyari*. In every other instance where flies of this age were used the results were negative. They would either appear frightened or show no interest whatever in the caterpillars before them.

OVIPOSITION

Oviposition has been repeatedly observed. The fly faces the host, and, with its ovipositor extended between its legs, calmly proceeds to attach an egg to the skin of the host larva. Often the fly will stand at almost the maximum distance away from the host larva, so that an active larva could easily crawl out of range. This behavior was apparent when the species was confined with *Hyphantria cunea* Drury or *Diacrisia virginica* Fab. Oviposition was frequently attempted on the rapidly moving larvae, but rarely were the flies able to deposit their eggs. As a rule, once a larva escaped, the parasite would not only fail to follow it up, but apparently lost all interest in it. With a less active species of host, especially the tenthredinids, the flies were more persistent and deposited many eggs. In certain species of this family a larva when disturbed raises the anterior portion of its body and jerks its head backwards and sideways until relieved from alarm. This behavior on the part of the species observed was futile, however, so far as *Sturmia inconspicua* was concerned. Such action may have delayed oviposition, but eventually the flies succeeded in their attack and deposited many eggs.

The fertilized female, when given preferred host material, did not exhibit the degree of excitement noted among some other tachinids. This was also true of the females that had been held back after fertilization for experimental purposes. Only rarely did any of the flies exhibit excessive eagerness to oviposit, even under what were considered to be ideal conditions.

Sensing of the host by tapping it with either the front feet or the antennal organs was not a prerequisite to attack, although the flies would often go through with these preliminaries. Motion, even of inanimate material, seemed to supply the necessary stimulus, and occasionally they would attempt oviposition on the forceps or brush as it was being withdrawn from the vial which contained them.

Oviposition was obtained at all hours of the day. Night conditions were simulated as nearly as possible, but the flies that would oviposit by day would not do so in darkness.

Apparently the flies will deposit their eggs on any part of the host. Perhaps the pleural region received the most eggs. If so, it was simply because this region was most accessible. As a rule, the eggs were laid perpendicular to the longitudinal axis of the host larva, although they were frequently found otherwise. Eggs deposited on the head capsule and on the dorsum where the integument is thickest have been noted. When they are laid on the head it is not known to a certainty that the maggots succeed in penetrating. If not, they probably die, for in no instance has any migration of the maggot been observed. A maggot hatching from an egg laid on the dorsum of *Neodiprion dyari*

or *Porthetria dispar* has no difficulty in penetrating. In other species the maggot might experience some difficulty, but no information concerning this point is available. At the Budapest laboratory, when eggs were laid on the proleg of *P. dispar*, the maggots were successful in reaching the body cavity. Eggs deposited on the sides of the thoracic segments have the best chance of survival, as the host larva is then unable to crush them. No data are available, however, which could possibly be interpreted to indicate that the fly was aware of this fact.

In confinement the number of eggs deposited on a single host is governed largely by the fly's eagerness to deposit or by the length of time that the host is subject to attack.

Sturmia inconspicua shows a preference as to its hosts. This is illustrated by the following experiment: Larvae of various stages of *Porthetria dispar*, *Malacosoma americana* Fab., *M. disstria* Hbn., *Hemerocampa leucostigma* A. and S., and *Neodiprion dyari* were placed in a cage with a number of *S. inconspicua* and allowed to remain until the next day. Subsequent examination of this material showed all the larvae free of eggs except the *N. dyari*, which were heavily infested. Other experiments were of a confirmatory nature. For instance, if *inconspicua* were placed in the same cage with *Diprion simile* and *N. dyari*, their interest would eventually center upon the latter. Furthermore, flies actively engaged in attacking *N. dyari* when transferred to *P. dispar* or *Malacosoma* spp. failed to exhibit the same eagerness to oviposit. When these hosts were replaced by *N. dyari*, the flies resumed their former interest.

LONGEVITY

Under laboratory conditions *Sturmia inconspicua* is a hardy species, there being little difficulty in keeping the flies in good condition. Individuals well cared for by daily feeding and cleansing of containers lived for 4 or 5 weeks. An instance was observed in which two females, removed from the mating cages, in coitu with males, on May 31 and used daily in reproduction experiments, lived for 42 days. A case of one female fly living for 112 days has been recorded at the Budapest laboratory.

PARTHENOGENESIS

Virgin females showed little interest in the usual hosts and, although they occasionally attempted oviposition, they were never successful. Furthermore, dissection of the flies revealed no egg development whatever. Parthenogenesis is therefore unlikely. The fact that no infertile eggs were deposited by any of the flies is interesting. In *Carcelia laxifrons* Vill., *Phorocera agilis*, and other tachinids, even though the female is unfertilized, the eggs will descend into the uterus and eventually be deposited.

DEVELOPMENT OF THE LARVA

From egg deposition to the complete disappearance of the maggot within the host is a matter of 5 to 10 minutes only. The maggot hatches immediately from one end of the egg and bores its way into the caterpillar, the chorion of the egg collapsing simultaneously with the exit of the maggot. Its progress is then arrested and it soon

becomes enveloped within an integumental funnel caused by an ingrowth of the body wall of the host. Apparently there is no attachment to any of the tracheae, but the maggot obtains its supply of air directly from the exterior by way of the funnel, in which its posterior stigmata are tightly fitted. This funnel is cone-shaped, the basal portion being highly sclerotized, dark brown to black, and gradually fading out into a lighter shade anteriorly until it becomes hyaline and difficult to see. The entire first and second stages and a part of the third stage are spent in this fixed position. The maggot then leaves its funnel and wanders freely in the body of the caterpillar. It is thought to remain thus for at least a day. This belief is strengthened by the fact that, when nearly mature third-stage maggots are dissected out alive and are allowed to remain on a watch glass with the dead host material, they will feed and complete their development and produce perfect adults. Under such conditions several maggots have lived for more than a day before transforming to pupae.

Sometimes the attack of the parasite does not bring about any definite reaction from the host larva other than a slight contraction of the segments at the moment of oviposition. Certain individuals seem to be totally unaware of the entrance of the maggot and display no apparent discomfort. Others move constantly about, throwing back the head and trying to dislodge the burrowing maggot. A parasitized larva, when exposed to a second attack one to seven days later, behaves precisely as does one suffering its initial attack. Apparently there is no recognition of its enemy.

The time required for the growth of the different larval stages is variable. Over 100 penultimate-stage larvae of *Neodiprion dyari* were parasitized in one day. Dissections of this material, together with that obtained from other host species (*Malacosoma disstria*, *M. americana*, and *Porichetria dispar*), showed a great disparity in larval development. First-stage maggots were found, either alone or in company with more fully developed ones, in larvae that had been parasitized up to 12 days; second-stage maggots were found in material parasitized from 6 to 12 days; and third-stage maggots in material parasitized from 6 to 27 days. The average duration of the larval stages of 104 individuals was 13.9 days. Usually it was not until the sixth day that any stage other than the first was found, although a few individuals reached maturity in this time. There seems to be no extra development of the maggot in the uterus of females that have withheld their eggs for an extended period. Some of the flies were held in reserve and not allowed to oviposit until 27 days after fertilization, but their progeny did not develop any more rapidly than those that were treated normally. From all the available data a mean of 3 to 6 days has been calculated as best suited to the larval development in each stage. These figures will account for at least 90 per cent of the larvae observed. It is not believed that an equal time is spent in each stage; in fact, there is some evidence that the second stage is shorter than either of the other two.

THE PUPAL PERIOD

Rarely does the maggot form its puparium within the host. Usually it cuts its way through the end of the cocoon or, in the case of a lepidopteran, tears its way through the skin of the host larva, crawls

a short distance away, and pupates. In the rearing trays the parasites often formed their puparia in the excrement and other débris. Owing to this habit and also because of the spiny nature of their skin, to which all small particles will adhere, the puparia are often mistaken for other matter and thus are easily overlooked.

The duration of the pupal stage of the summer generation was found to average about 10.5 days. A rare exception was a minimum of 7 days. The maximum time recorded was 18 days, but no doubt this could be extended almost indefinitely by lowering the temperature. The time spent in the puparium seems to depend somewhat on the sex of the individual. Of the 68 flies studied, 32 males averaged 10.9 days and 36 females averaged 11.3 days.

HIBERNATION

Sturmia inconspicua hibernates as a first-stage maggot in the cocooned larva of *Lophyrus pini* and in the overwintering larva of *Dendrolimus pini*. At the Budapest laboratory it has been observed that when the cocoons of *pini* are opened, and the overwintering larvae removed, those which are parasitized may be readily detected by the protuberance caused by the heavily sclerotized breathing funnel of the parasite. Often this structure is surrounded by a dark brown scab, and close examination reveals a minute hole through which the posterior spiracles of the parasite maggot are discernible. Communicating with the outside in this way, the *Sturmia* larva passes the winter in a well-defined sac which is attached to the sides of the funnel.

At the Melrose Highlands laboratory *Sturmia*-infested overwintering hosts (*Diprion simile* and *Neodiprion lecontei* Fitch) were dissected in October and in February of the next year, and without exception first-stage *inconspicua* maggots were found encysted beneath the skin of the host larva at their point of entrance, in a manner as described above.

SUPERPARASITISM

Superparasitism is often encountered in dissection work. Several examples which may be of interest are as follows: One *Porthetria dispar* larva parasitized 6 days contained one second-stage and one third-stage maggot; one larva of *Neodiprion dyari* parasitized 6 days contained one first-stage and four second-stage maggots in various stages of development; one *P. dispar* larva parasitized 10 days contained one second-stage (newly molted) and three third-stage maggots; one *N. dyari* parasitized 12 days contained one first-stage and one third-stage maggot.

This excessive parasitism is not always advantageous to the parasite. Many of the *Neodiprion dyari* larvae were infested with more than one parasite, yet the actual rearing of two parasites from one individual was not recorded. Evidently, in this host at least, it is the one parasite best fitted that survives. From *Porthetria dispar*, *Malacosoma americana*, and *M. disstria* several parasites have frequently been obtained from a single individual. From the Budapest laboratory Muesebeck reports three to five parasites from a single *P. dispar* larva as common, and as many as seven have been obtained. With these species it is probable that the number of *inconspicua* in each caterpillar is limited only by the size of the host.

HYPERPARASITES

According to Sitowski,⁷ *Sturmia inconspicua* suffers from two hyperparasites in Poland, (*Hemipenthes*) *Anthrax morio* L. and *Argyrotaenia varia* Fab. The parasitism by the former is sometimes severe. At the gipsy moth laboratory there is no record of any secondary parasite having been reared from material imported from Europe.

COMPETITION WITH OTHER PARASITES

This important phase of parasitism has not been studied in this country with sufficient thoroughness to warrant anything more than a general statement. In Europe, at the Budapest laboratory, some of the most interesting observations on the competition occurring between *Sturmia inconspicua* and other parasites have been made by C. F. W. Muesebeck and his assistants. These observations⁸ show that—

* * * *Sturmia inconspicua* is able to complete development more quickly and thus win out in competition with two of the most common of our central European parasites of *Porthetria dispar*. When eggs of *Sturmia* are laid on *dispar* larvae that were parasitized as many as 8 days previously by *Phorocera agilis* or 5 days previously by *Tachina larvarum* L. it is usually the *inconspicua* larvae which first complete development and form puparia. *S. inconspicua*, when developing in otherwise parasite-free stock, requires from 6 to 20 days to form puparia, whether or not more than one *inconspicua* larva is present. *P. agilis* requires from 17 to 26 days and *T. larvarum* from 9 to 20 days under the same conditions. Since the average *P. agilis* larva requires more time than the average *Sturmia*, it is easily understood why *inconspicua* larvae issue first, but the case of *T. larvarum* is different. *T. larvarum*, on an average, requires no more time than *S. inconspicua*, most of its larvae forming puparia on the eleventh day in the laboratory. Nevertheless, *Sturmia* regularly wins over this species in competition, and it seems as if the presence of *inconspicua* larvae must have some retarding influence on those of *T. larvarum*. Later in the season *inconspicua* completes development in the larval stage very much more rapidly than *T. larvarum*. Parasite-free *dispar* larvae attacked by *T. larvarum* on August 30 produced no puparia until October 2, 33 days later, whereas *S. inconspicua* needed but from 9 to 20 days in larvae attacked September 21. In the summer, though, at the regular *dispar* season, the two species develop separately in about the same time. When, on the other hand, 30 *dispar* were attacked first by *S. inconspicua* and later by *P. agilis*, probably only the larvae which would never have produced *inconspicua* puparia or which supported both species produced those of *agilis*. Thirty *inconspicua* puparia were reared from this lot, all but one of which issued before a single one of the *P. agilis* puparia which were obtained.

From the foregoing it is evident that, because of its rapid development, *Sturmia inconspicua* is well able to hold its own even when competing against two of the most important parasites of *Porthetria dispar*.

In the United States *Porthetria dispar* has no native parasites worth mentioning. Of the introduced parasites that have been successfully established, only three, which attack the larger caterpillars, will be of interest here. These are *Compsilura concinnata* Meig., *Sturmia scutellata*, and *Phorocera agilis*, tachinid flies from Europe. The effect on these species of the establishment of *S. inconspicua* is problematical. So far as *Compsilura* is concerned, it is probable that an intensive competition would result in which *Compsilura* would be the loser. Should this prove to be the case, the status of

⁷ SITOWSKI, L. DO BIOLOGII PAROZYTOW BORECZNIKA (LOPHYRUS LATR.). (SUR LA BIOLOGIE DES PARASITES DE LOPHYRUS LATR.) Roczn. Nauk Rolnicz. 14: 1-25, illus. 1925. [Original in Polish; title in both Polish and French. Summary in German, Zur Biologie der Lophyrusparasiten, p. 22-25.]

⁸ Unpublished notes at the Budapest laboratory.

Compsilura would not be materially affected because of its extraordinary ability to adapt itself to other hosts. On the other hand, *S. scutellata* and *P. agilis* are peculiar to *P. dispar*; moreover, their development is comparatively slow. For these reasons it is probable that on occasions of periodic abundance of *inconspicua* they would indeed suffer. It has often been stated that the natural control of *P. dispar* in the European forests is due, in part, to the large number of species that are parasitic upon it. Here the struggle for supremacy, or perhaps for the perpetuation, of the parasitic species themselves, is as acute as in the United States, if not more so, and yet these species persist. It is safe to predict that the establishment of *inconspicua* in this country would in no way interfere with the continuance of the other species.

The parasites of *Diprion simile* have been recorded by Middleton.⁹ Mention is made of seven hymenopterous parasites and one tachinid, *Phorocera claripennis* Macq. (*Exorista petiolata* Coq.). Two species of Hymenoptera, *Monodontomerus dentipes* Boheman and *Ephialtes* (*Itopectis*) *conquisitor* Say, and two tachinids, *Phorocera claripennis* Macq. and *Winthemia quadripustulata* Fab., have been reared at the gipsy moth laboratory. In no case were these parasites abundant. From collections of approximately 1,000 larvae only five individuals were parasitized, and it seems unlikely that *inconspicua* would find much competition in the United States as regards this host.

It is difficult to conceive of any instance in which the native parasites would suffer because of *Sturmia inconspicua*. Certainly other species besides *Porthetria dispar* and *Diprion simile* will be attacked, and the parasites peculiar to them must suffer the competition of *inconspicua*. It is by no means certain, however, that such competition would jeopardize the species so much as do numerous other factors which exert their influence entirely independent of host or parasite. The author knows of no instance in which a foreign tachinid has usurped the position of a native one. *Compsilura concinnata* is a far more adaptable species than *S. inconspicua*; yet, with all its reputed aggressiveness, there is little indication that its presence has in any way proved detrimental to its native competitors.

HOSTS

A list of the European hosts of *Sturmia bimaculata* Htg. (= *S. inconspicua* Meig.) prepared by Baer¹⁰ includes the following species: *Dendrolimus pini* L., *Lymantria* (= *Porthetria*) *dispar* L., *L. monacha* L., *Panolis griseovariegata* Goeze, *Stauropus fagi* L., *Lophyrus pini* L., *L. similis* Htg., *L. pallidus* Klug, *L. virens* Klug, *L. frutetorum* Fab., *L. socius* Klug, *L. rufus* Klug, *L. polytomus* Htg., and *L. laricis* Jurine.

At the Budapest laboratory *Sturmia inconspicua* has been reared from several additional hosts, which are as follows: *Trichiocampus viminalis* Fall., *Pygaera pigra* Hufn., *Plusia gamma* L., *Agrotis c-nigrum* L., and from two unidentified tenthredinids.

Other host species, exclusive of *Porthetria dispar* and *Diprion simile* Htg., which have been reared at the Melrose Highlands laboratory

⁹ MIDDLETON, W. THE IMPORTED PINE SAWFLY. U. S. Dept. Agr. Bul. 1182, p. 16, illus. 1923.

¹⁰ BAER, W. Op. cit.

are *Malacosoma americana* Fab., *M. disstria* Hbn., *Hemerocampa leucostigma* A. and S., *Hydria undulata* L., *Alypia octomaculata* Fab., *Neodiprion dyari* Roh., and *Neodiprion lecontei* Fitch.

SUMMARY AND CONCLUSIONS

Sturmia inconspicua Meigen is an effective European parasite of the gipsy moth, *Porthetria dispar* L. It is also the most important European tachinid parasite of the introduced pine sawfly, *Diprion simile* Htg., and because of the recent introduction of this species in the Northeastern States the establishment of *S. inconspicua* should prove of decided benefit.

European authorities do not wholly agree upon the taxonomic status of *Sturmia inconspicua*, but according to J. Villeneuve the form treated in this paper is that species.

Sturmia inconspicua is indigenous to central Europe and is commonly found in northern Africa. The first attempt to colonize the species in the northeastern part of the United States was in 1906. Between 1907 and 1910 other introductions were made, but during the following years all attempts to recover the species failed. In more recent years (1923 to 1925, inclusive) large colonies were liberated and in 1929 the first recovery was made.

Superficially, the adult bears a general resemblance to the common house fly, but on critical examination it will be found to possess certain characters that are typical of the genus *Sturmia*, family Tachinidae. The eggs are grayish and about 0.5 mm in length. The first-stage larva is white, more or less spinose, and characterized chiefly by the presence of stigmatic spines and sclerotized areas on the posterior segment. The bucco-pharyngeal armature is without articulation. The second-stage larva appears less spinose and lacks the two principal characteristics of the preceding stage. The bucco-pharyngeal armature has one articulation. The third-stage larva is characterized by a clothing of spines and long hairs and protuberant posterior spiracles. The bucco-pharyngeal armature has two articulations. The puparium is brownish black, averaging 4 or 5 mm in length, and has tuberculate spiracles each furnished with three spiracular slits.

The species passes the winter as a first-stage maggot within the cocooned larvae of several species of sawflies of the old genus *Lophyrus* and possibly in some lepidopterous pupae. Completing its development in the spring of the year, the maggot issues, forms its puparium, and in about two weeks the adult emerges. There is a second generation, the species being able to develop on a number of hosts. It is the progeny of this generation which hibernate.

The species will readily mate in confinement. Ordinarily the flies remain in coitu several hours. Males frequently remate and the result of the second union is successful.

The preoviposition period is variable, 12 to 14 days usually being required.

The fly attaches its eggs to the skin of the host larva. In confinement the number of eggs deposited on a single caterpillar is governed largely by the eagerness of the individual to oviposit or by the length of time that the host is subject to attack.

Under laboratory conditions the flies will live for 4 or 5 weeks. An instance of one female living for 112 days has been recorded.

The species is believed not to be parthenogenetic.

The average duration of the entire larval stage is about 13.9 days, although certain individuals will mature in 6 or 7 days. The duration of the pupal stage averages about 10 days.

Superparasitism is frequent, as many as seven individuals having been obtained from a single caterpillar.

In Europe two species of hyperparasites attacking *S. inconspicua* are known, but none has been recorded from the United States.

Competition with other parasites is certain to occur, but because of the rapid development of *S. inconspicua* it will probably not suffer in this respect.

In addition to *Porthetria dispar* and *Diprion simile*, *Sturmia inconspicua* has been reared from many other host species, seven of which are native to the United States.

CORRELATIONAL AND ALLIED STUDIES OF THE PROTEIN CONTENT, WATER ABSORPTION, LOAF VOLUME, AND LOAF WEIGHT OF TWO SERIES OF HARD RED SPRING WHEATS¹

By L. R. WALDRON, *Agronomist (Plant Breeding)*, and C. E. MANGELS, *Cereal Chemist, North Dakota Agricultural Experiment Station*²

INTRODUCTION

This paper presents a comparison between two series of hard red spring wheats. One series consisted of wheats diverse in genetic origin but grown under uniform conditions of soil and soil treatment. The second series consisted of a single variety grown on the plots of a rotation and fertility experimental project, located on a single soil type (Fargo clay) but subjected annually to diverse previous treatments relative to crop sequence and soil fertilization. This paper is concerned with the behavior of the two series in regard to the means, coefficients of variability, correlations (both total and net), and regression equations relative to the characters of (1) protein content, (2) water absorption of flour, (3) volume of loaf, and (4) weight of loaf.

REVIEW OF PREVIOUS INVESTIGATIONS

A number of statistical studies, emphasizing correlation results in particular, have been published on wheat for milling and on flour during the past decade. The studies reported, however, have not usually compared different series of data with their appropriate statistical constants. No doubt this is largely due to the fact that wheat and flour data have generally accumulated in connection with other experimental work and have been used only secondarily for statistical studies. Studies of variation between groups of data have thus not been possible because of limitations in number and suitability. Attention will be paid to such a comparison in this paper.

The first important studies on wheat and flour were those of Zinn (18)³ who used the published data from a number of experiment stations. Zinn evidently appreciated the limitations of the data with which he worked and called particular attention to the importance of their proper grouping.

Bailey and Hendel (1) in their studies on the relation between protein content and test weight per bushel called attention to the necessity of grouping data by crop years, and this point was emphasized also by Mangels and Sanderson (11) and by Mangels (8).

Because of the importance of protein content as a price factor, particular attention has been given to the protein-loaf volume relation. A summary of protein-loaf volume correlation coefficients obtained by different investigators for hard red spring wheats is given in Table 1.

¹ Received for publication Jan. 26, 1932; issued September, 1932.

² The authors acknowledge permission to use the data from the rotation and fertility plots kindly given by Dr. H. L. Walster, chairman of the Agronomy Department and in charge of the rotation and fertility project; also their indebtedness to Dr. A. E. Treloar and his coworkers for the use of material from their study prior to its publication.

³ Reference is made by number (italic) to Literature Cited, p. 230.

TABLE 1.—*Protein-loaf volume correlation coefficients obtained by different investigators from hard red spring wheats*

Investigator	Description of wheat	Coefficient
Zinn (15).....	North Dakota commercial varieties.....	* -0.099
	North Dakota pure lines.....	.302
	Minnesota commercial varieties.....	.259
	Minnesota pure lines.....	.547
Mangels (8).....	Montana commercial varieties.....	.345
Bailey and Sherwood (2).....	North Dakota miscellaneous named varieties.....	† .334
	Diverse wheats from Minnesota, North and South Dakota, and Montana.....	.271
Hayes, Immer, and Bailey (6). Coleman, Dixon, and Fellows (5).....	Diverse varieties and hybrid selections.....	† .280
	Varieties diverse as to locality and variety.....	† .432
Larmour (7).....	Standard commercial varieties.....	† .280
Harris (6).....	Marquis wheat of the 1920 Saskatchewan crop.....	† .670
Treloar et al. (12).....	Commercial samples milled by Minnesota State testing mill.....	† .490

* Zinn evidently included durum wheats which accounts for the negative coefficient. Recalculation of data for the hard red spring wheats only by Mangels (8) gave a positive coefficient of 0.523.

† Weighted average of 1,807 samples for 11 crop years separately calculated. Maximum coefficient 0.547, minimum coefficient -0.064.

* Weighted average of coefficients for 4 crop years separately calculated. Maximum coefficient 0.50. Each milled sample consisted of a mixture of 4 wheats, of the same kind, grown at 4 Minnesota localities.

† Average of coefficients from crops of 1923 and 1924.

* Three crop years, 1926, 1927, and 1928. Bromate baking formula gave coefficient of 0.711.

† Bromate formula gave a coefficient of 0.857.

* Weighted average of 332 samples for the 6 years, 1921-1926.

The correlation coefficients between protein and loaf volume are not usually of high order. With the exception of Zinn's comparison of pure lines and commercial varieties of North Dakota and Minnesota spring wheats, no direct comparisons are found between different groups of samples. Most of the correlation coefficients reported were obtained from groups of samples diverse within the group as to variety, and often also in other respects. The highest coefficient is that reported by Harris (6) for a group of samples consisting only of Marquis wheat secured from the 1929 Saskatchewan crop.

With the exception of the attention given to the subject by Treloar and others (12), comparatively little emphasis has hitherto been placed on the regression coefficients, in studies of the relationship of loaf volume to protein content. It has been assumed that a uniform relationship exists between the degree of correlation and the volume of the loaf. The relationship of this kind is not uniform but shows decided variations. The prediction value, deducible from the relationships of protein and volume, is decidedly affected by the deviations among the variates as shown by the regression coefficients and even then it may be necessary to consider additional variables, which find expression in the partial regression coefficients. This subject will receive further attention.

METHODS

The two series of wheats previously mentioned covered four years of cropping. One series, which consisted of wheats of somewhat diverse genetic origin was grown in the wheat-breeding nursery under uniform conditions each season. This series is designated as "nursery." The second series, which consisted of a single variety of wheat (Ceres) grown each year on a number of rotation and fertility plots diverse as to crop sequence, soil preparation, and use of fertilizers, is designated as "plots."

Since both series were grown at the North Dakota Agricultural Experiment Station, not more than one-half mile apart and also on the same type of soil (Fargo clay), they were subjected to similar climatic conditions.⁴

Three of the characters already mentioned are quantitative—protein, loaf volume, and loaf weight. The fourth character, water absorption, is expressed quantitatively, but the quantity used is estimated.

The flours used in all these studies were 75 per cent patents, prepared on a small mill. The baking tests were made according to the procedure recommended by the American Association of Cereal Chemists, using a variable absorption.

STATISTICAL CONSTANTS AND COEFFICIENTS OF VARIABILITY

The means, standard deviations, and coefficients of variability for the two series of wheats for the four years are shown in Table 2.

TABLE 2.—*Statistical constants secured from two diverse series of wheats, from the nursery and from the plots, 1927-1930*

MEANS						
Year	Nursery or plots	Protein of flour *	Water absorption of flour	Loaf weight	Loaf volume	Variates
		Per cent	Per cent	Grams	C c	Number
1927	Nursery.....	13.1±0.06	63.3±0.17	136.4±0.20	415.0±2.11	76
	Plots.....	10.4±.06	61.1±.13	135.4±.22	373.0±1.29	51
1928	Nursery.....	14.8±.09	61.2±.13	131.1±.24	454.0±4.92	59
	Plots.....	12.2±.09	55.2±.04	129.0±.18	484.0±4.89	70
1929	Nursery.....	10.8±.07	57.1±.14	133.3±.18	410.0±2.95	81
	Plots.....	12.1±.03	60.7±.05	136.0±.15	415.0±1.35	80
1930	Nursery.....	13.4±.07	58.5±.10	131.8±.25	466.0±4.23	58
	Plots.....	13.1±.10	63.7±.05	137.4±.10	464.0±2.29	58
STANDARD DEVIATIONS						
1927	Nursery.....	0.75±0.04	2.22±0.12	2.58±0.14	27.1±1.49
	Plots.....	.66±.05	1.37±.09	2.29±.15	13.6±.91
1928	Nursery.....	1.00±.06	1.52±.10	2.69±.17	55.6±3.48
	Plots.....	1.06±.06	.47±.03	2.20±.13	60.2±3.46
1929	Nursery.....	.85±.05	1.88±.10	2.32±.12	39.1±2.09
	Plots.....	1.01±.05	.69±.04	1.91±.10	17.8±.95
1930	Nursery.....	.76±.05	1.18±.07	2.81±.18	47.7±2.99
	Plots.....	1.14±.07	.61±.04	1.13±.07	25.9±1.62
COEFFICIENTS OF VARIABILITY (IN PERCENTAGE)						
1927	Nursery.....	5.70±0.31	3.50±0.19	1.89±0.10	6.55±0.36
	Plots.....	6.37±.43	2.24±.15	1.69±.11	3.64±.25
1928	Nursery.....	6.74±.42	2.48±.16	2.05±.13	12.25±.87
	Plots.....	8.86±.51	.85±.05	1.71±.10	12.43±.72
1929	Nursery.....	8.62±.46	3.29±.18	1.74±.09	9.54±.51
	Plots.....	8.38±.45	1.14±.06	1.41±.08	4.28±.23
1930	Nursery.....	5.65±.36	2.01±.13	2.13±.13	10.24±.65
	Plots.....	8.73±.55	.96±.06	.82±.05	5.58±.35

* Protein determinations were made with the water content calculated to a uniform basis.

The nursery group of wheats varied in its constituents somewhat from year to year. During the first three years a considerable proportion of the wheats were hybrid selections of the cross Marquis × Kota, sibs of Ceres. The remaining wheats were named varieties and selections from various crosses. The Marquis × Kota hybrids showed less variability in protein content than did the other wheats,

⁴ In 1928 the two lots were affected differently by rain after cutting and previous to threshing. (Table 4.)

but a decrease in the proportion of these wheats for 1930 did not result in a relative increase of variability of protein for that year. Any increase in variability that may have occurred from a diminution in number of Marquis \times Kota hybrids must have been offset by decreases from other causes.

PROTEIN CONTENT OF FLOUR

Protein content may be measured with a relatively higher degree of accuracy than other characters, and on account of its importance will be discussed separately.

The average unweighted protein content for the two series for the four years was 13 per cent for the nursery and 12 per cent for the plots. This very appreciable difference may be due wholly or in part to the previous cropping of the nursery wheats. This was as follows:

Year	Previous cropping
1927,	summer-fallowed sweetclover turned under.
1928,	flax preceded by sweetclover turned under.
1929,	oats preceded by sweetclover turned under.
1930,	cucurbits preceded by wheat, flax, and summer-fallowed sweetclover turned under.

Sweetclover preceded the wheat in all cases, but immediately only in 1927. The 1928 crop of oats carried a heavy growth of straw, and unavoidably a considerable amount of stubble was plowed under. There seems to be no reasonable doubt but that the nitrogen available to the 1929 wheat crop was greatly influenced by the crop of oats. The probable effect of the preceding oat crop on the protein content of the 1929 nursery wheat is indicated by the protein present for the years other than 1929. The average percentages of protein for the years 1927, 1928, and 1930, as compared with those for 1929 are as follows:

	Nursery	Plots
Average for 1927, 1928, 1930.....	13.8	11.9
Average for 1929.....	10.8	12.1
Difference.....	3.0	-0.2

Data from rotation plots (Mangels (9)) show that a preceding crop of sweetclover on Fargo clay soil increases the protein content of the succeeding wheat crop. Further evidence of the influence of preceding crops is shown by the protein content of the Ceres samples from the nursery samples and the Ceres averages for the plots. These comparisons are given in Table 3.

TABLE 3.—*Protein content of Ceres wheat when grown in the nursery and in the plots, compared with the protein content of all nursery wheats*

Year	Protein content of all wheats grown in nursery (1)	Protein content of Ceres grown in—		Differences between—	
		Nursery (2)	Plots (3)	1 and 2	2 and 3
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>		
1927.....	13.1	12.9	10.4	0.2	2.5
1928.....	14.8	14.9	12.2	.1	2.7
1929.....	10.7	10.2	12.1	.5	1.9
1930.....	13.4	13.1	13.1	.3	0
Average.....	13.0	12.8	12.0	.28	1.78

The protein of Ceres in the nursery samples is much closer to the general nursery average than to the average of the plots series consisting entirely of the Ceres variety.

The means of the characters other than protein are very close together. The unweighted means of water absorption, loaf weight, and loaf volume for the four years were 60, 133.2, and 436, respectively, for the nursery, and 60.2, 134.5, and 434 for the plot wheats.

Attention should be called to that portion of Table 2 showing the coefficients of variability. The comparative behavior of protein under modified conditions indicated previously is shown in the present instance. To give a clear expression to the relative differences of behavior of this character in comparison with water absorption, loaf weight, and loaf volume, the differences of variability calculated from Table 2 are shown in Table 4.

TABLE 4.—*Differences of the coefficients of variability between the nursery and plots from Table 2 for the four characters indicated, 1927-1930*

[When the ratio values (*R*) are 3 or more the differences may be considered significant]

Year	Protein content *	Water absorption	Loaf weight	Loaf volume *
1927	-0.67±0.53; <i>R</i> =1	1.26±0.24; <i>R</i> =5	0.20±0.15; <i>R</i> =1.3	2.91±0.44; <i>R</i> =7
1928	-2.12±.66; <i>R</i> =3.2	1.63±.17; <i>R</i> =10	.34±.16; <i>R</i> =2.1	6-.18±1.13; <i>R</i> =0.2
1929	.24±.64; <i>R</i> =.3	2.15±.19; <i>R</i> =11	.33±.12; <i>R</i> =2.8	5.26±.56; <i>R</i> =9
1930	-3.08±.66; <i>R</i> =4.7	1.05±.14; <i>R</i> =7	1.31±.14; <i>R</i> =9.4	4.66±.74; <i>R</i> =6

* Minus differences indicate that the variability of the plots is more than that of the nursery samples.

^b The slightly greater variability in loaf volume for the plots in 1928, in sharp contrast to that obtained for the other years, may easily be due to a disturbing element of that year. The wheat from the plots was subjected to heavy rains while in the shock, whereas the nursery samples were subjected to little or no extraneous moisture before threshing.

Considering the coefficients of variability for proteins, one finds that the variability of the plots is greater in three of the four years. In two of these three years (1928 and 1930) the variability of the protein content from the plots is significantly the larger.

A closer study of the data on protein content shows that the nursery samples exhibited greater variability than would be expected from a group of Ceres samples taken at random. In 1927 and 1928 more than half of the nursery samples were sibs of Ceres, resulting from the same cross, while the remainder were miscellaneous in character. The coefficients of variability for these two groups for the two years were calculated and are shown in Table 5.

TABLE 5.—*Coefficients of variability for protein content of nursery samples that were sibs of Ceres as compared with those that were miscellaneous in character, 1927 and 1928*

Kind of samples	Variability of protein content in—	
	1927	1928
Miscellaneous.....	8.53±0.80	7.51±0.78
Sibs of Ceres.....	3.41±.23	3.84±.24
Difference.....	5.12±.83	3.67±.82

The protein content of the miscellaneous wheats when grown under uniform conditions is seen to be decidedly more variable than the Ceres sibs similarly grown. However, the Ceres variety, grown on the rotation and fertility plots, shows more variability among the different samples than do the samples from the nursery.

Another comparison may be made by securing differences between the coefficients of variability of the protein content of the Ceres samples from the plots and of the Ceres sibs from the nursery, for 1927 and 1928. The values are shown in Table 6.

TABLE 6.—Coefficients of variability for protein content of Ceres samples from the plots as compared with samples from nursery Ceres sibs, 1927 and 1928

Kind of samples	Variability of protein content in—	
	1927	1928
Ceres from plots.....	6.37±0.43	8.86±0.51
Ceres sibs from nursery.....	3.41±.23	3.84±.24
Difference.....	2.96±.49	5.02±.56

The evidence for the greater variability of the protein content of the samples from the plots is very definite for three of the four years. For 1929, when wheat followed oats, the difference, which is negative, is not significant. In this case the nursery samples had a relatively high standard deviation associated with a comparatively low mean.

So far as the present studies show, the preparation of the soil, crop sequence, and use of fertilizers, generally, have a greater influence on the protein content of wheat than do differences in genetic origin of wheat. Differences in protein due to genetic origin, while important, evidently are not responsible for as much variation as differences in soil fertility.

LOAF VOLUME, LOAF WEIGHT, AND WATER ABSORPTION

The means of loaf volume, loaf weight, and water absorption show no uniformity as to differences in value between the nursery and the plots, but the coefficients of variability are almost uniformly larger for the nursery samples as shown in Table 4. Only one exception is found in the 12 possible comparisons. The coefficient of variability for loaf volume for the plots for 1928 is slightly the greater, but this is probably due to sprout damage in the rotation plot samples. In 8 of the 12 cases, the differences are strikingly significant.

A distinct difference in variability is found between protein and the other three characters. Protein content responds readily to soil differences, whereas water absorption, loaf weight, and loaf volume respond to varietal differences. Of the three characters, loaf weight, loaf volume, and water absorption, loaf weight responds least and water absorption most, to varietal differences.

CORRELATION DATA

Correlation coefficients were computed between the variables for the two series. In addition to the total correlation, partial correlations were also calculated. Evidently very little use has been made

of partial correlation coefficients when studying wheat and flour data. These data seem to offer an excellent field for the application of the partial correlation. Certainly the problem allows closer approach when, for example, a direct relationship can be established between protein and loaf volume without the interference of such variables as water absorption and loaf weight, which for this purpose can be made constant.

The entire series of partial correlations are presented. A number of these may possess only minor interest, but they are listed since the general problem demanded their calculation. The coefficients of the zero, first, and second order are shown in Table 7. The weighted means of the correlation coefficients, calculated by the method of Fisher (4, p. 170), are presented.

TABLE 7.—Total and partial correlation coefficients and weighted averages secured from two diverse series of wheats, from the nursery and the plots, calculated from the four characters shown, 1927-1930

[Correlations given in columns 4, 5, and 6, represent values when element, or elements, at head of the columns was held constant]

PROTEIN-VOLUME						
Year	Nursery or plot	Zero order	Loaf weight	Water absorption	Weight and absorption	Variates
1927	(Nursery.....	0.506	0.496	0.508	0.480	76
	(Plots.....	.683	.716	.683	.745	51
1928	(Nursery.....	.351	.350	.262	.155	59
	(Plots.....	.712	.616	.711	.616	70
1929	(Nursery.....	.322	.307	.337	.298	81
	(Plots.....	.674	.684	.666	.676	80
1930	(Nursery.....	.583	.430	.578	.367	58
	(Plots.....	.850	.853	.848	.852	58
Average, nursery.....		.441	.398	.427	.337	.274
Average, plots.....		.735	.722	.732	.726	.259

PROTEIN-WEIGHT						
Year	Nursery or plot	Zero order	Loaf volume	Water absorption	Volume and absorption	
1927	(Nursery.....	-.0.124	-.0.044	-.0.239	-.0.147	
	(Plots.....	-.125	-.316	-.221	-.456	
1928	(Nursery.....	-.056	.047	-.278	-.182	
	(Plots.....	-.488	-.200	-.486	-.199	
1929	(Nursery.....	-.101	-.011	-.195	-.108	
	(Plots.....	-.334	-.365	-.334	-.365	
1930	(Nursery.....	-.438	-.102	-.499	-.134	
	(Plots.....	-.148	.200	-.139	.207	
Average, nursery.....		-.174	-.028	-.294	-.140	
Average plots.....		-.301	-.190	-.316	-.245	

PROTEIN-ABSORPTION						
Year	Nursery or plot	Zero order	Loaf volume	Loaf weight	Volume and weight	
1927	(Nursery.....	0.077	0.092	0.219	0.167	
	(Plots.....	.078	.067	.198	.352	
1928	(Nursery.....	.412	.344	.481	.381	
	(Plots.....	-.056	-.027	-.007	-.009	
1929	(Nursery.....	.198	.223	.257	.246	
	(Plots.....	-.138	-.018	-.138	-.018	
1930	(Nursery.....	.271	.254	.363	.281	
	(Plots.....	.273	.244	.269	.250	
Average, nursery.....		.253	.239	.321	.263	
Average, plots.....		.027	.056	.056	.120	

TABLE 7.—Total and partial correlation coefficients and weighted averages secured from two diverse series of wheats, from the nursery and the plots, calculated from the four characters shown, 1927-1939—Continued

ABSORPTION-WEIGHT

Year	Nursery or plot	Zero order	Protein content	Loaf volume	Protein and volume
1927	Nursery	0.673	0.690	0.682	0.690
	Plots	.617	.634	.618	.676
1928	Nursery	*.422	*.489	*.552	*.571
	Plots	.103	.087	.088	.085
1929	Nursery	*.382	*.412	*.387	*.399
	Plots	.022	-.026	.004	-.003
1930	Nursery	.122	*.278	.283	*.307
	Plots	-.053	-.013	-.002	-.054
Average, nursery		.437	.494	.499	.514
Average, plots		.162	.157	.164	.168

ABSORPTION-VOLUME

Year	Nursery or plot	Zero order	Protein content	Loaf weight	Protein and weight
1927	Nursery	-0.005	-0.052	0.152	* 0.051
	Plots	.042	-.015	-.005	-.303
1928	Nursery	*.298	.180	*.479	*.379
	Plots	-.053	-.018	.000	.006
1929	Nursery	-.040	-.111	.077	-.002
	Plots	-.185	-.126	-.184	-.123
1930	Nursery	.112	-.058	.279	.160
	Plots	.172	-.118	.165	-.129
Average, nursery		.076	-.021	.233	.133
Average, plots		-.026	-.074	-.034	-.127

VOLUME-WEIGHT

Year	Nursery or plot	Zero order	Protein content	Water absorption	Protein and water absorption
1927	Nursery	-0.172	* -0.127	* -0.227	* -0.127
	Plots	.151	.326	.159	.434
1928	Nursery	-.282	-.280	-.471	-.428
	Plots	.516	-.275	-.514	-.275
1929	Nursery	-.263	* -.265	-.290	* -.243
	Plots	-.097	.183	-.095	.182
1930	Nursery	* -.700	-.531	* -.724	* -.618
	Plots	-.283	-.320	-.288	-.324
Average, nursery		-.362	-.293	-.426	-.344
Average, plots		-.219	-.027	-.216	-.004

* These pairs show significant differences. Any values of the weighted means numerically larger than 0.130 may be considered to show a significant difference.

† Total.

The coefficients in Table 7 are not accompanied by their probable errors. In lieu of giving the probable errors, 5 *P* values for *n*=45, 50, 60, 70, and 80 from Table V. A. of Fisher (4) are shown in Table 8.

TABLE 8.—Correlation coefficient values for selected levels of significance (from Fisher) for coefficients shown in Table 7

	<i>P</i> =0.1	<i>P</i> =0.05	<i>P</i> =0.02	<i>P</i> =0.01
45	0.243	0.288	0.338	0.372
50	.231	.273	.322	.354
60	.211	.250	.295	.325
70	.195	.232	.274	.302
80	.183	.217	.257	.283

In using these P values it must be remembered that n represents the net number of variates after deduction has been made from n' (= total variates) to secure the proper number of degrees of freedom. For the total correlation $n = n' - 2$ and with each variable held constant, an additional unit is taken from n' , this last-named value equaling the total variates involved. Simple interpolation can be used for in-between values of n . Coefficients with values larger than those indicated naturally have P values less than those shown and are of greater significance. The pairs of correlations that have differences with P values of 0.05 or less (Table 7, footnote *a*) show significant differences.

PROTEIN-VOLUME COEFFICIENTS

Most of the zero-order protein-volume coefficients are fairly high. The correlation is uniformly higher for the plots, and in three out of four cases the difference is large enough to be significant.

Coefficients of correlation previously reported in the literature for the protein-volume relation have been summarized in Table 1. The average of the nursery for four years is 0.441, which is somewhat higher, but comparable to, the coefficients summarized in Table 1. The average of the plots, however, is 0.735, which is considerably higher than other coefficients reported, with the exception of the value 0.670 reported by Harris (5).

Coefficients of correlation which have been calculated from diverse strains of varieties and without regard to crop year probably do not represent as sharp reactions between protein and loaf volume as one finds in commercial mills where one wheat variety from one crop very often dominates the blend of a mill mix.

If the variety factor is held constant, therefore, as in the plots, there is a relatively high degree of correlation, averaging 0.735, between protein and loaf volume. This correlation for the plots would be sufficiently high to justify the payment of substantial premiums for protein content. The question arises in this connection whether these samples have been subjected to as much diversity as would a series of samples taken from a commercial mill. In the present instance the run of the weather for parts of four years is represented, rather than that from geographical weather variations of a single year, which one would look for in a commercial lot. In a commercial lot, also, a diversity of soil types and an appreciable amount of varietal difference would affect the degree of correlation between protein and loaf volume.

Not only are the zero-order protein-volume coefficients of the plots decidedly larger than those from the nursery, but their value is maintained better when the other two components, weight and water absorption, are held constant, either singly or together. In case of samples from the nursery, weight and water absorption contribute a significantly larger amount to the total correlation than do the samples from the plots. In the latter case also, the average decrease in coefficient values for the 12 possible comparisons is but 0.008 in comparison with the corresponding average of 0.060 for the nursery correlation. The difference for these two values is significant. When weight and water absorption are held constant, the 4-year average change from the total correlation for the plots is 0.008 as compared with 0.116 for the nursery.

When a single variety is grown under diverse conditions, the total protein-volume correlations are less affected, in this comparison, by holding weight and absorption constant than when several varieties of wheat are grown under the same conditions. This is merely a suggestive conclusion, as there are too few comparisons for significance.

VARIATION IN WHEAT PROTEIN-VOLUME AND FLOUR PROTEIN-VOLUME CORRELATION

Zinn (13) found high correlations between protein of wheat and protein of flour. Treloar and others (12) determined the correlation coefficient between the protein in the flour and in the wheat and found a weighted average value of 0.948 for a period of six years. The correlation coefficients between these two characters and loaf volume ran closely parallel for the various years, the two weighted averages not showing a significant difference. In such cases, it would evidently matter but little whether the correlation dealing with loaf volume was concerned with the wheat or with the flour protein. Since the flour used in this case was a comparatively short patent (75 per cent) it seemed advisable to determine correlations in regard to this point. Wheat-protein data were lacking for the nursery samples for 1927, and results are presented for three years only in Table 9.

TABLE 9.—Correlation coefficients between protein in wheat and protein in flour and the correlation of each of these with loaf volume for the samples from the nursery and the plots, 1928-1930 *

Characters correlated	Correlation coefficients in—					
	1928		1929		1930	
	Nursery	Plots	Nursery	Plots	Nursery	Plots
Protein in wheat and in flour.....*	0.953	0.978	0.827	0.980	0.807	0.988
Protein in wheat and loaf volume.....	.324	.777	.330	.648	.473	.854
Protein in flour and loaf volume.....	.351	.712	.322	.674	.583	.850

* The significance of these correlation values can be obtained from a reference to Table 8.

The correlations between protein in wheat and protein in flour are lowest in all cases for the nursery samples. Differences are strongly significant for 1929 and 1930. It seems rather likely that correlations between the protein content of wheat and flour are greater where only one variety is concerned, even though grown under diverse conditions, than where several varieties are involved, even when these are grown uniformly.

For 1930 the correlation between wheat protein and loaf volume is appreciably lower than that between flour protein and loaf volume for the nursery plots. In 1929, however, the correlation for the nursery samples between wheat protein and volume is slightly higher than that between flour protein and volume.

In 1928 a lower correlation was found between flour protein and loaf volume for the plots, but the reverse was true in 1929. From the foregoing, one can not conclude that there is any great advantage in using the flour-protein figure as compared with wheat protein even though the correlation between the two protein contents is not extremely high in some cases.

CORRELATION OF PROTEIN TO WEIGHT

The zero-order correlations are negative in all cases, but only in three of eight cases are the coefficients significant. The changes, from the total to the partial coefficients when volume or absorption, or both, are held constant, are of no particular moment. Generally there is a slight tendency for loaf weight to be depressed with an increase of protein. For the plots, 1928, a coefficient of -0.488 is shown. When loaf volume, or loaf volume and water absorption are held constant in this case, the coefficient is reduced to -0.200 and -0.199 , respectively. The plot samples for 1928 contained sprouted wheat which likely tended to increase volume, on account of increased rate of fermentation, with a consequent tendency to lower the loaf weight.

CORRELATION OF PROTEIN TO ABSORPTION

The correlation coefficients between protein and absorption are not striking, although the majority are positive. The changes from the total to the net correlations are unimportant. The correlations for the nursery samples are positive throughout, while for the plot samples there are two positive and two negative coefficients. This would indicate that the relation between these two characters is more consistent when the series is diverse as to variety and genetic origin. The variation between different crop years is quite large.

Correlation results for the three characters volume, weight, and water absorption, in relation to protein, show that striking results are found only in the protein-volume relationship. Loaf weight and water absorption evidently are not closely related to protein content, and other factors apparently have greater influence on these two characters than has protein content.

CORRELATION OF WATER ABSORPTION TO WEIGHT AND VOLUME

Considering the relationship of water absorption to loaf weight, diverse results are encountered, and only in 1927 does one find comparatively high total correlations. For the nursery, all four of the zero-order coefficients are positive, and three are significant. For the plots, three coefficients are positive and one negative, but only one coefficient is significant. Mangels (10) reported coefficients of correlation for absorption and weight for four crop years the average of which was 0.730 . Mangels used a series of wheats of different varieties from crops of 1923 to 1926, inclusive. The corresponding coefficients from the nursery samples for four years averages 0.437 , which is significantly lower. This difference may perhaps be ascribed to seasonal variation. The partial correlations do not show appreciable differences from the zero-order coefficients.

The absorption-volume correlations are of comparatively little importance since in only a few instances do they become significant. These results are similar to those obtained by Mangels (10) for crops of 1923 to 1926, except that the coefficients reported by Mangels are positive in all cases. Comparing the zero-order coefficients for the nursery samples and for the plots, both series show two negative and two positive coefficients. When protein and weight are held constant, however, the nursery data show three positive and one negative coefficient for the four years, while the plots show three negative and one positive coefficients.

CORRELATIONS OF VOLUME AND WEIGHT

The correlations under this heading are of greater interest, generally, than those of the four preceding groups. Evidently not much attention has been paid to this phase of correlation, for no results seem to be reported in the literature. Only 6 of the 32 coefficients are positive, and but 2 of these are significant. Eighteen of the 26 negative coefficients are significant. It is evident that the relationship between volume and weight is not directly operative or organic. If the loaf weight tends to be depressed with an increase in size, then this relationship must be referred to protein or to absorption, or to both, in this present study. Considering this particular negative correlation the question of linearity immediately arises; there is evidently a limit to the continuous separation of these two characters. By taking the total correlation for the 1930 nursery samples, which lies at -0.7 , a table was made showing the distribution of the variates. This is shown in Figure 1. Within the limits of the indicated class values the evidence for nonlinearity is not apparent. Fisher's test (4, p. 219) was applied, and the difference z , indicating lack of linearity, was wholly inappreciable. With this result the writers calculated the partial coefficients, which would have been a questionable procedure with much curvilinear regression.

		Weight of loaf (grams)																	
		126	127	128	129	130	131	132	133	134	135	136	137						
Volume of loaf (cubic centimeters)	385							1				1			2				
	395							1	1						2				
	405										1	2	1		4				
	415						1				1				2				
	425							1	1		1	2			5				
	435								1	2	1				4				
	445					2		2	2						6				
	455					1					1				2				
	465				1	2								1	5				
	475									1					5				
	485						3	1	1						4				
	495			1				1	2						2				
	505				1				1						4				
	515		1	1		1	1								3				
	525				1		1	1							1				
	535	1													1				
	545	1													2				
	555		1		1										2				
	565		1				1								1				
	575			1			1								1				
		2	3	3	4	6	9	10	6	8	5	5	2	58					

FIGURE 1.—Correlation diagram showing relationship between volume and weight of loaf for 58 variates from the 1930 nursery samples. The regression is linear and the correlation is -0.7

With the negative correlation between volume and weight it may be inferred that in those loaves in which the diastatic activity was greatest, with a resultant large gas production, a comparatively large loss of weight automatically took place. This loss in weight is presumed to be due largely to the destruction of carbohydrates by the yeast. In studying the volume-weight correlations one is struck by their lack of uniformity, either with regard to conditions of growth or to the individual year. When water absorption is held constant the changes from the total correlation are only slight. When protein is held constant, the changes from the total to the partial correlation are mainly positive and are distinctly significant.

Considering the correlation relations of the six groups of Table 7 only three, those concerned with protein and volume, absorption and weight, and volume and weight, have coefficients sufficiently large to make the correlations important. The correlations of the protein-volume group are the largest and most uniform. In general, the changes in size of coefficients, from the zero order to the partial, are not uniform within a group and are not of much importance.

Interesting as the foregoing results may be, it should not be forgotten that the correlation values in themselves do not exhibit the full measure of their importance. It is necessary to consider the regression coefficients, calculated from the standard deviation and the coefficients of correlation. From the regression coefficients it is possible to estimate deviations in loaf volume, for instance, according to any arbitrary deviation in protein.

REGRESSION COEFFICIENTS

While the total regression coefficients are worked out very simply according to the formula $b = r_{12} \frac{\sigma_1}{\sigma_2}$, the partial regression coefficients are calculated by a set of normal equations. An outline of the method is given by Fisher (4, p. 133-135). In calculating these, attention was paid mainly to the regression of volume on the two characters, protein and loaf weight. The values for absorption were usually not significant. The total and partial regressions expressed in cubic centimeters of loaf are shown in Table 10.

TABLE 10.—Total regression coefficients of loaf volume on protein and on loaf weight and partial regression coefficients of volume on protein and on weight, with respect to absorption and weight, and to absorption and protein

[Values are expressed in cubic centimeters of loaf]

Year	Nursery or plot	Protein		Weight	
		Total	Partial	Total	Partial
1927.....	(Nursery.....)	*18.42	*17.35	—1.81	—1.62
	(Plots.....)	*14.12	*14.99	.90	*1.60
1928.....	(Nursery.....)	*19.53	8.30	*—5.83	*—9.37
	(Plots.....)	*39.51	*33.36	*—14.12	*—6.33
1929.....	(Nursery.....)	*13.59	*12.53	*—4.77	*—4.24
	(Plots.....)	*11.81	*12.42	— .90	1.31
1930.....	(Nursery.....)	*36.65	*18.17	*—11.88	*—10.11
	(Plots.....)	*19.24	*19.07	*—6.68	*—3.91

* These values are distinctly to highly significant. The other values are not significant, or scarcely so.

The values in Table 10 are to be interpreted as loaf-volume deviations in cubic centimeters for every respective unit deviation in protein or weight. The total regressions are calculated with regard to only two characters, whereas the partial regressions take account of all four characters. The difference between the total and the partial correlations is only slight in some cases but very considerable in others. Where one finds pronounced differences the partial coefficient is usually the smaller. A comparison between these coefficients and the correlation values of Table 7, made by averaging the total and second-order correlations and the total and partial regression coefficients for the four years, is shown in Table 11.

TABLE 11.—*Weighted averages of correlation and regression coefficients taken from Tables 7 and 10 for samples from nursery and from plots for the two relationships: Protein-volume and weight-volume*

Characters correlated	Total				Partial			
	Correlation		Regression		Correlation		Regression	
	Nurs- ery	Plots	Nurs- ery	Plots	Nurs- ery	Plots	Nurs- ery	Plots
Protein-volume.....	0.441	0.735	21.41	25.60	0.337	0.726	14.15	20.07
Weight-volume.....	-.362	-.219	-5.60	-3.77	-.344	-.004	-5.86	-1.87

Table 11 shows briefly what has been indicated previously. Under "total" for protein-volume, the two regressions differ by about 4 units, but the two correlations differ rather markedly. Under "partial" the two correlations differ still more, the regression average from the plots being nearly 6 units more. For weight-volume the nursery correlations are appreciably the larger in both cases; the partial regression values show more difference than is found between the total regression values. In brief, the plot samples show a direct relationship when volume is considered with protein, but with regard to the relationship of weight to volume the nursery samples show the higher values, either positive or negative.

It has been suggested to the writers by Doctor Treloar of the University of Minnesota that it is possible to combine into one correlation table paired data extending over a series of years (or events) with a correlation coefficient equal to the weighted mean coefficient secured by averaging the coefficients of the individual years. In order to do this it is necessary to eliminate the disorderly differentiation found from season to season due to differences in means and the corresponding disorderliness due to the various series of deviations from the different means. This is done by securing ratios of each series of deviations to the corresponding standard deviations. All standard deviations are thus reduced to unity. This was done with the plot and nursery samples for the four years with regard to protein and loaf volume. The two results, for the relationship of protein and volume, are as follows:

Nursery, 1927-1930.....	0.439
Plots, 1927-1930.....	.711

The results are shown in Figures 2 and 3. As the standard deviation values are unity, the correlation coefficients become the regression

values. In considering values like the one just presented, their limitations must be strictly considered. They can not be used for prediction purposes but may be used in a comparison of two or more sets of data combined into one set, with a similar combination. In a comparison of this sort differences may be observed in the slopes of the two lines present and also the disposition of the means of the arrays of each of the two sets. Considering the two correlation surfaces, one can compare the disposition of the two groups of variates. In Figures 2 and 3 the slope of the regression line is greater in Figure 3, the approximation of the means of the arrays to the regression line is greater in this figure, and finally the compactness of the variate groups shown in Figure 3 is greater. The ratio of the two slopes is 1.6. It will be recalled that the variability of protein was appreciably greater for the samples from the plots than for the nursery samples. This confirms what has already been indicated, that a series of protein values have greater influences on the loaf volume when they are restricted as to genetic origin, although unrestricted as to cultural conditions. Comparatively high variability in the protein content need not operate against high prediction values with respect to the volume of the loaf.

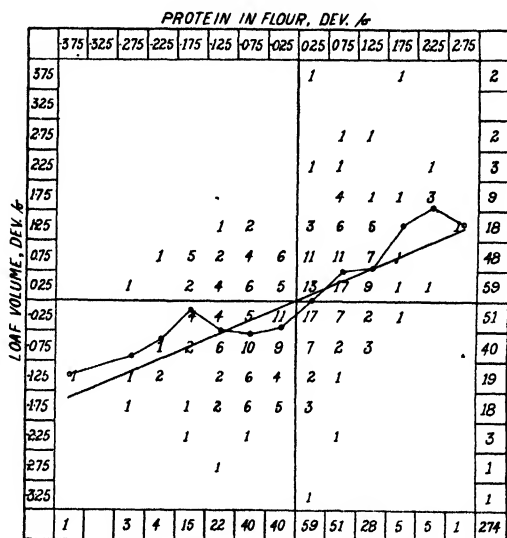


FIGURE 2.—Correlation diagram showing relationship between loaf volume and protein content of wheat from the nursery for 1927 to 1930. Differentiation of the means and standard deviations from year to year are eliminated by using the ratios indicated. The sloping line is the regression of the loaf volume on protein content expressed in ratios and is equal to the weighted mean of the four annual coefficients of correlation, which is equal to 0.439. The standard deviations are each equal to unity

APPLICATION OF REGRESSION COEFFICIENTS

A clearer idea is gained of the meaning and application of the regression coefficients if they are applied directly to the values concerned. In Table 12 the various regression formulas are shown. In addition the various expected loaf volumes are calculated, assuming the flour protein content to be uniformly 12 per cent. This protein percentage for flour is higher than that generally found in commercial flours but is one-half per cent lower than the average of the flours dealt with here. The ranges of loaf volumes are shown within which 80 per cent of the loaves are expected to fall according to a normal distribution. The theoretic ranges are compared with the actual, and the number of loaves coming under, and also over, the theoretic limits are indicated.

TABLE 12.—Regression formulas derived from total regression coefficients with ranges of loaf volume expected to include 80 per cent of the variates, assuming a protein content of 12 per cent for each year, and the number of variates falling below and above the theoretic limits

Year	Nursery or plot	Formula (volume=)	Number of variates	Mean		Most likely volume at 12 per cent protein	Range within which 80 per cent of variates are expected	Actual protein content minus assumed value	Variates outside theoretic limits	
				Protein	Volume				Under	Over
1927	Nursery	18.42 protein + 173.4	76	13.1	415	394	359-429	+1.1	0	20
	Plots	14.12 protein + 226.4	51	10.4	373	396	370-413	-1.6	34	0
1928	Nursery	19.53 protein + 164.9	59	14.8	454	399	328-470	+2.8	3	34
	Plots	39.51 protein + 1.0	70	12.2	484	477	400-554	+2.2	1	10
1929	Nursery	13.59 protein + 263.3	81	10.8	410	426	370-476	-1.2	13	3
	Plots	11.81 protein + 272.4	80	12.1	415	414	391-437	+1.1	5	7
1930	Nursery	36.65 protein - 25.5	58	13.4	466	414	353-475	+1.4	0	22
	Plots	19.24 protein + 211.9	58	13.1	464	443	410-476	+1.1	0	17
1928	Nursery	*8.30 protein + 331.1	59	14.8	454	431	360-502	+2.8	5	7
	Plots	*18.17 protein + 222.1	58	13.4	466	440	379-501	+1.4	0	30

* As above with 2 partial regression formulas derived from partial regression coefficients.

Table 12 shows that the number of loaves under and over the theoretic limits is anything but uniform. Obviously this is due to

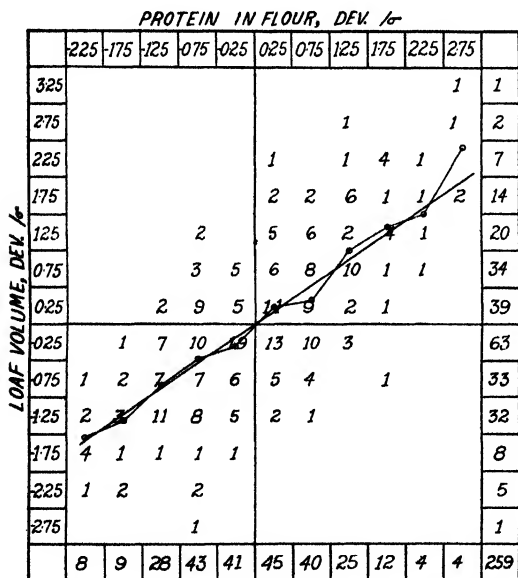


FIGURE 3.—Correlation diagram showing relationship between loaf volume and protein content of wheat from the plots for 1927 to 1930. Differentiation of the means and standard deviations from year to year are eliminated by using the ratios indicated. The sloping line is the regression of the loaf volume on protein content expressed in ratios and is equal to the weighted mean of the four annual coefficients of correlation, which is equal to 0.711. The standard deviations are each equal to unity

results are not presented, except the two referred to, which are shown at the bottom of Table 12. The number of loaves outside the lower and upper limits are more equalized for the 1928 nursery sam-

ple. As the assumed 12 per cent protein content is below the average of 12.5 per cent, the number of loaves over the maximum limit might be expected to be larger than those under the minimum limit and this is actually the case, the difference being 57. The number of loaves under and over the limits of 80 per cent is considerably in excess of the theoretical 20 per cent, and this is also due to the arbitrary placing of the protein per cent.

The partial regression formulas were also calculated; and as they gave nearly identical expected loaf volumes (with two exceptions), the

ples, using the partial regression coefficients, but are less uniform for the 1930 nursery samples. The various limits within which 80 per cent of the variates are presumed to fall are in direct proportion to the respective standard deviations of loaf volume. This is evident from the fact that the latter are multiplied by a constant abscissal value x distant ± 1.28 from the central ordinate of the normal curve.

In order to make a comparison of prediction values of the two series, weighted means were taken of both the total and partial regression equations. This appears to be a legitimate procedure, as these equations contain essentially only means and correlation coefficients, which are both subject to weighted means. In taking the weighted means of the standard deviations the variances rather than the standard deviations were weighted. The following equations were thus obtained:

Weighted-mean total-regression equations

Nursery: Volume = 21.41 protein + 162.3
Plots: Volume = 25.60 protein + 128.7

Weighted-mean partial-regression equations

Nursery: Volume = 14.15 protein + 251.5
Plots: Volume = 20.07 protein + 193.2

When the two pairs of regression equations are calculated, the differences in the coefficient portions are nearly the same. The protein coefficient for the plots is larger in each case and probably significantly so. The difference between the coefficients is only slightly larger in the case of the partial correlations. These differences, though perhaps not of great importance, show the protein of the one variety, grown on the plots, to be the more potent in its effect on loaf volume. It is obvious that regression lines, calculable from the equations shown above, would scarcely be applicable to a correlation surface. As the correlation surfaces shown in Figures 2 and 3 deal with ratios and not with actual values, the regression lines from the above equations, which deal with actual values, could not be drawn on the surface nor used for prediction purposes in connection with ratios. If the paired values between protein and volume for the four years of a series were to be entered on a correlation surface the "disorderly differentiation," appearing when seasonal results are compared, discussed by Treloar and others (12), would dominate the situation and would not accord with the weighted regression lines. In such a case, also, the individual years would not be handled as units.

DIVERSE SEASONAL EFFECTS

Seasonal conditions have a marked influence, as is well known, on the amount and quality of protein. Table 2 shows that the amount of protein from the plot samples is essentially the same for the two years 1928 and 1929. Loaf volume, on the contrary, showed nearly three times as much variability for 1928 as for 1929, a relatively enormous difference. The correlation coefficients for the two years, however, did not show a significant difference. These facts, along with the regression equations, are shown in Table 13.

TABLE 13.—Means of protein, variabilities of loaf volume, coefficients of correlations, and regression equations to show seasonal diversity of protein effect in two successive years, shown by plot samples

Year	Protein	Variability of volume	Coefficients of correlation, (P-V)	Regression equation (volume=)	Volume deviation in cubic centimeters per units of protein
	Per cent	Per cent			
1928	12.2	12.43	0.712	39.51 protein+1.....	40
1929	12.1	4.28	.674	11.81 protein+272	12

The prediction value of the protein as to loaf volume is more than three times greater for 1928. The foregoing clearly indicates that

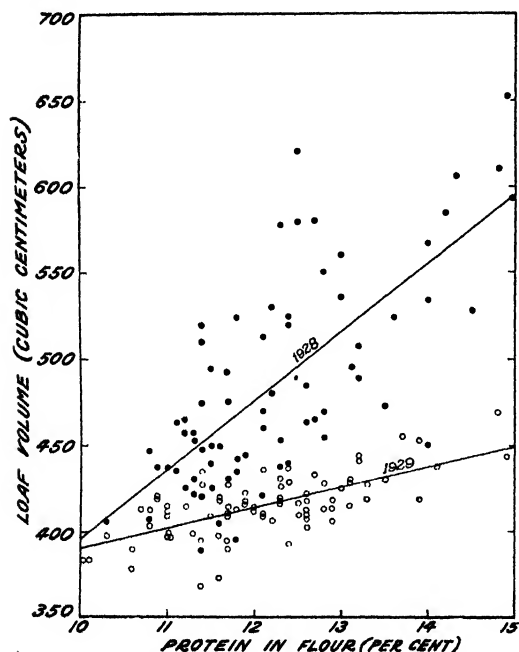


FIGURE 4.—Scatter diagram showing relationship between loaf volume and protein content of flour for the wheat samples from the plots for two successive years. The regression coefficients for 1928 and 1929 are 39.5 and 11.8, respectively

neither the amount of protein nor the degree of correlation between protein and loaf volume is always a dependable criterion as to the market value of protein. This remarkably different behavior of the protein relative to loaf volume that may occur from a single variety in two different seasons grown upon a group of plots with diverse manurial and crop sequence treatments is presented in Figure 4. This is a scatter diagram of loaf volume and flour protein for the two years with the two regression lines superimposed.

DISCUSSION

In the production of a loaf of bread there is involved a highly complicated set of physical and chemical reactions. The basic material, flour, is a colloid mixture in a dry condition, necessarily complicated, to which is added other substances, water, minerals, yeast, fat, and sugar, previous to subjection to heat, to produce the finished loaf. An immense number of loaves must be made, and these must be made from flours ground from various grades and classes of wheats. A desirable loaf must fall within rather narrow limits to satisfy the public taste and fashion.

As a consequence the millers and bakers seek for a single criterion, or for only a very few criteria, to apply to the flour in evaluating the future loaf. Because of its unique properties, protein in wheat flour has received a great deal of study. This substance is considered so important that variations in wheat prices are based on the amount of protein in many cases. This being the case, it is evident that a very full knowledge of the protein content of the wheat and the resulting flour is demanded. This knowledge is of a twofold nature: On the one hand, a study is needed of the relationships affecting the laying down of the protein in the wheat kernel during the formative period, with regard to variety and the total environment, and on the other hand a study is needed of the relationships of the protein, as laid down, to the loaf in its various stages. While considerable knowledge of this sort is already available, a satisfactory goal has not been reached.

In the present paper, data are presented from two series of hard red spring wheats which throw some additional light upon the protein problem. The nursery series, grown under comparable conditions, varied genetically, while the plots series, grown under varying conditions, consisted of samples genetically uniform. The higher-average protein content shown by the nursery samples, as an average for the four years, may be ascribed very likely to sweetclover turned under, preceding the wheat crop by one or two years. In 1929 when the protein content for the nursery samples was lower than that of samples from the plots, oats preceding the nursery wheat evidently caused the low protein content. Evidence is presented to show the much greater influence exerted on the protein content by soil variability, developed in the rotation and fertility plots, than by genetic differences present in the nursery samples. Evidence for this is very positive for three of the four years but neutral for the fourth year.

If the two series of wheats are compared as two units and not by years, the variability of the protein from the plots is found to be much the greater. The coefficients of variability for the two series are: Plots 18.43 ± 0.56 , and nursery 12.56 ± 0.37 . The difference is 5.87 ± 0.67 which is highly significant. This is a different sort of a comparison, however, than when the comparative variability is considered year by year.

Variability of the protein content of a series of wheat samples is apparently due more to the result of external conditions, soil and climate, than to the genetic character of the wheat. Put in another way, a series of protein values will show greater variability when affected by a random sampling of external conditions than when affected by a random sampling of internal (genetic) conditions.

A discussion of climate in the present paper has been almost entirely omitted as it was believed that a consideration of that factor would complicate the discussion more than was desirable. It is not believed that weather conditions differed enough to affect the comparisons. Either of the two series, and especially the plot series, affords excellent material to study the effect of weather on the amount and character of protein.

With the characters of water absorption, loaf volume, and loaf weight, the effect is generally contrary to the finding for protein, for in these cases genetic differences are responsible for a strikingly greater degree of variability than are the environmental conditions, diverse

as to soil fertility and crop sequence. It is fair to presume that a similar statement would hold if a comparison were made between genetic differences and climatic environmental conditions. It is evident from this that although the amount of protein may show marked variability, this variability need not be much reflected in the variability of loaf characters.

Correlation data were calculated between the four characters, protein, water absorption, loaf volume, and loaf weight, for the samples from the nursery and from the plots for the four years under consideration. The correlation coefficients showing the relationships between protein and volume are uniformly higher for the samples from the plots where one variety was grown than from corresponding samples from the nursery, which were genetically diverse. Evidently where diverse strains or varieties enter into the correlation study, one does not find so sharp reactions between protein and loaf volume as are found in commercial mills where the flour output consists of, or is dominated by, one variety. The correlations between protein and volume were distinctly higher than between any other pair. Correlations were positive and significant in all cases. In the five remaining cases the coefficients were only occasionally significant for the samples from the nursery or from the plots for the four years. For two series of correlations, those showing relationship between protein and weight and between volume and weight, the coefficients were almost uniformly minus, and in a few cases rather distinctly significant. Evidently fermentative activities which tend to produce large volumes do so at the expense of some of the carbohydrate material in the loaf. In one instance where the coefficient between volume and weight was -0.70 the regression was essentially linear for the class limits which existed. In this extreme case, where the loaf showed the greatest loss in weight, associated with large volume, it was found that the weight of the loaf suffered about 1.5 per cent, using the standard deviation of loaf volume as the criterion.

The net or partial correlation coefficients were calculated. In most of the cases the change from the total to the net correlations is not marked. In only a few cases was the change significant. In the other relationships the total correlation coefficients were generally low, and no marked change in absolute values would be expected in comparing total and net coefficients.

It is not evident from the data that there is any great advantage in using the flour protein in obtaining the correlations even though correlations between wheat protein and flour protein are not extremely high. Treloar and others (12) held wheat protein and flour protein alternately constant and correlated for the other character. In doing this it became evident that the wheat protein was a better criterion of loaf volume than the flour protein. They conclude that those wheats of the hard red spring class which have a higher protein concentration in the bran than in the endosperm are of somewhat better baking quality relative to loaf volume. In the present study, with only three years' data available, higher correlations between wheat protein and flour protein seem to exist where only a single variety is concerned rather than with many varieties.

In calculating the total and partial regression coefficients of volume on protein, significant amounts were found in practically all cases. In fact, the value of this relationship was rather dominating relative

to other relationships. Figure 4 shows the marked difference there may be as to the effect of protein on the loaf from one season to another, irrespective of the quantity of the protein and with but little regard to the degree of correlation. In following out the idea of Treloar by eliminating the disorderliness appearing in the data from season to season, correlation surfaces are obtained easily comparable with one another and whose "regression lines" are equal to the respective weighted correlation coefficients. Although the ratio of the two slopes of the nursery samples to the plot samples was 1.6, this does not express the relative difference in the prediction value of the protein to loaf volume. When the two weighted regression coefficients are calculated, for the nursery and for the plots, the difference between them is relatively low, in contrast to the rather marked difference between the two correlation coefficients. This evidently arises from the facts already emphasized—that while the variability of the protein from the plots is relatively high, the variability of the loaf volume (and also water absorption and loaf weight) is relatively low.

SUMMARY

Two series of hard red spring wheats are compared in regard to the means, coefficient of variability, correlations (total and net), and regression equations relative to the character of protein content, water absorption of flour, loaf volume, and loaf weight. One series, designated as nursery, consisted of wheats diverse in genetic origin but grown under uniform conditions of soil and soil treatment, while the other consisted of a single variety grown on plots that were subjected to different treatment in regard to crop sequence and soil fertilization.

The flour-protein content of the nursery samples was higher than for the plot samples for the four years, which was evidently due to the fact that sweetclover had been plowed under previous to the wheat. The unweighted means of water absorption, loaf weight, and loaf volume are very close together; the small differences show no significances.

The protein content showed significantly greater variability for three years for the plots and a less, but nonsignificant, variability for one year. Considering the two series as units, the variability of the protein from the plots showed distinctly greater variability for the four years, the difference being 5.87 ± 0.67 .

In contrast to the above, the nursery samples showed moderately to distinctly greater variability in 9 of the 12 cases where water absorption, loaf weight, and loaf volume were concerned. In only one instance was the variability greater for the plot samples.

Varied soil and crop-sequence conditions acting on a genetically uniform wheat resulted in greater protein variability than did genetically diverse wheats under uniform conditions. Genetic variability, on the other hand, expressed through uniform soil conditions, resulted in greater variability for water absorption, loaf weight, and loaf volume in comparison with a single variety grown diversely as to soil fertility and crop sequence.

Complete total and partial correlation coefficients were secured. The protein-volume correlations were of greatest moment, and those from the plots were higher than from the nursery samples in all cases

and significantly so in 11 of the 16 comparisons. This leads to the conclusion that a more direct relationship exists between protein and loaf volume in a commercial mill, where the wheat of one variety very often dominates the blend in a mill mix, than in a genetically diverse group of wheats commonly grown experimentally.

Correlations of protein and water absorption and water absorption and volume were generally small and not significant. There was no significant relationship of loaf weight to absorption relative to the plot samples, but this relation generally was moderately significant so far as the nursery samples were concerned. Considering the relationships of protein and weight and volume and weight, the correlations were generally negative and of distinct significance in certain instances. In the extreme case, with a correlation of -0.7 between volume and weight, a loss of weight of 1.5 per cent would be expected as a result of baking. Within the class limits present, the foregoing negative relationship was linear.

In most cases the changes in value, from total to net correlations, were not of much importance.

For three years, where data were available, the correlations between protein in wheat and loaf volume and protein in flour and loaf volume showed no material differences.

Total and partial regression coefficients, with regard to regression of volume on protein, absorption, and weight, were calculated. The volume-absorption regressions were generally not significant. The volume-protein regressions were significant in practically all cases, both total and partial, and generally very distinctly so. The volume-weight regressions were negative in 13 out of the 16 cases, and 10 of the negative coefficients were significant. Seasonal effects showed markedly when the total regression equations were compared for two years from the plots. The loaf volume deviation in cubic centimeters for the deviation for each protein per cent was 40 for 1928 and 12 for 1929. Due to the variability in protein content from year to year, the annual range of loaves below and above the expected 80 per cent calculated from a uniform 12 per cent protein content was very unequal. The partial regression coefficients gave generally nearly the same prediction values for loaf volume based on protein as did the total regression formulas.

Correlation coefficients by themselves do not suffice to show to what extent loaf volume is dependent on protein content. Regression coefficients are necessary for a proper interpretation. In one comparison, with the two correlation coefficients nearly equal, a marked difference in loaf volume predictable from the protein was obtained from the results of two years. When correlation coefficients were significantly different, the regression coefficients at times approximated rather closely.

LITERATURE CITED

- (1) BAILEY, C. H., and HENDEL, J.
1923. CORRELATION OF WHEAT KERNEL PLUMPNESS AND PROTEIN CONTENT. *Jour. Amer. Soc. Agron.* 15:345-350.
- (2) ——— and SHERWOOD, R. C.
1926. RELATION OF CRUDE PROTEIN CONTENT OF FLOUR TO LOAF VOLUME. *Cereal Chem.* 3:393-401, illus.
- (3) COLEMAN, D. A., DIXON, H. B., and FELLOWS, H. C.
1927. A COMPARISON OF SOME PHYSICAL AND CHEMICAL TESTS FOR DETERMINING THE QUALITY OF GLUTEN IN WHEAT AND FLOUR. *Jour. Agr. Research* 34:241-264.

- (4) FISHER, R. A.
1928. STATISTICAL METHODS FOR RESEARCH WORKERS. Ed. 2, rev. and enl., 269 p., illus. Edinburgh and London.
- (5) HARRIS, R. H.
1930. RELATION BETWEEN CRUDE PROTEIN CONTENT AND LOAF VOLUMES OBTAINED BY TWO DIFFERENT METHODS OF BAKING. *Cereal Chem.* 7:557-570, illus.
- (6) HAYES, H. K., IMMER, F. R., and BAILEY, C. H.
1929. CORRELATION STUDIES WITH DIVERSE STRAINS OF SPRING AND WINTER WHEATS, WITH PARTICULAR REFERENCE TO INHERITANCE OF QUALITY. *Cereal Chem.* 6:85-96.
- (7) LARMOUR, R. K.
1930. RELATION BETWEEN PROTEIN CONTENT AND QUALITY OF WHEAT AS SHOWN BY DIFFERENT BAKING METHODS. *Cereal Chem.* 7:35-48, illus.
- (8) MANGELS, C. E.
1926. RELATION OF PROTEIN CONTENT TO BAKING QUALITY OF FLOUR FROM HARD RED SPRING AND DURUM WHEATS. *Cereal Chem.* 3:150-157, illus.
- (9) ————
1927. PRE-HARVEST FACTORS WHICH AFFECT WHEAT QUALITY. *Cereal Chem.* 4:376-388, illus.
- (10) ————
1928. RELATION OF WATER ABSORBING CAPACITY OF FLOUR TO PROTEIN CONTENT, BAKING QUALITY, AND LOAF WEIGHT. *Cereal Chem.* 5:75-77.
- (11) ———— and SANDERSON, T.
1925. THE CORRELATION OF THE PROTEIN CONTENT OF HARD RED SPRING WHEAT WITH PHYSICAL CHARACTERISTICS AND BAKING QUALITY. *Cereal Chem.* 2:107-112.
- (12) TRELOAR, A. E., ET AL.
1932. THE PREDICTION OF LOAF VOLUME. *Cereal Chem.* [In press].
- (13) ZINN, J.
1923. CORRELATIONS BETWEEN VARIOUS CHARACTERS OF WHEAT AND FLOUR AS DETERMINED FROM PUBLISHED DATA FROM CHEMICAL, MILLING, AND BAKING TESTS OF A NUMBER OF AMERICAN WHEATS. *Jour. Agr. Research* 23:529-548.

SOME MINOR STAINS OF SOUTHERN PINE AND HARDWOOD LUMBER AND LOGS¹

By T. C. SCHEFFER, *Agent*, and R. M. LINDGREN, *Assistant Pathologist, Division of Forest Pathology, Bureau of Plant Industry, United States Department of Agriculture*²

INTRODUCTION

In the course of experimental work on the control of sapwood stain and mold in southern pine³ and hardwood lumber and logs, various stains other than those of the ordinary blue-stain type were encountered frequently. Most of these ranged in color from bright yellow to deep red or purple, and under certain conditions were of sufficient intensity and prevalence to assume practical importance. In addition, certain common green surface molds were found to be capable of penetrating into pine lumber and producing a more or less shallow discoloration. Fresh pine sapwood stored under moist conditions was especially susceptible to stains and molds of this type. Such hardwoods as red gum,⁴ yellow poplar,⁵ and sweet bay,⁶ were generally free from them; but cultural tests in the laboratory showed a few of the organisms involved to be capable of staining sap gum, at least to a slight degree.

These stains appeared to be associated with and possibly stimulated by certain chemical solutions with which the lumber and logs had been treated for the purpose of preventing blue stain. Thus, the yellow and red discolorations were common on wood that had been immersed in formaldehyde, silicofluoride solutions, benzoic acid and its salts, arsenious oxide, or boric acid; a blue-green mold was prevalent on lumber treated with ethyl mercury compounds; while several green superficial molds were commonly associated with the use of sodium bisulphite, sodium silicate, ammonium fluoride, formaldehyde, and arsenious oxide. Although such relationships between treatments and organisms seemed to exist, they were not specific, as each of the stains was later produced on fresh untreated pine sapwood under proper conditions of moisture.

Stains of a similar nature have been described by Hedgcock⁷ in his study of chromogenic fungi that discolor wood. He found that certain *Penicillia*, principally *Penicillium aureum* Cda. and *P. roseum* Lk., commonly produce colors varying from yellow to crimson in the sapwood of pine and several hardwoods. In the case of *P. aureum*, the color was due to the secretion of a soluble pigment on the surface of the fungus filament. This pigment varied with the acidity or

¹ Received for publication Jan. 20, 1932; issued September, 1932.

² For their kindness in identifying the organisms discussed herein, thanks are due Dr. C. D. Sherbakoff, of the Department of Botany, University of Tennessee, and Dr. Charles Thom, Principal Mycologist in Charge, Division of Soil Microbiology, Bureau of Chemistry and Soils, U. S. Department of Agriculture.

³ The southern pine lumber referred to in this paper was derived from the following species: Longleaf pine (*Pinus palustris* Miller), shortleaf pine (*P. echinata* Miller), and loblolly pine (*P. taeda* L.).

⁴ The lumber known in the trade as red gum is derived from the sweetgum tree, *Liquidambar styraciflua* L.

⁵ The lumber known in the trade as yellow poplar is derived from the tuliptree, *Liriodendron tulipifera* L.

⁶ *Magnolia virginiana australis* Sargent.

⁷ HEDGCOCK, G. C. STUDIES UPON SOME CHROMOGENIC FUNGI WHICH DISCOLOR WOOD. Missouri Bot. Gard. Ann. Rpt. 17: 59-114, illus. 1906.

alkalinity of the culture media, being red when alkaline and yellow when acid, and was taken up by the cell walls of the wood. A third fungus, *Fusarium roseum* Lk., was identified as causing pink, red, and violet blotches upon fresh sapwood of *Pinus resinosa* Sol., *P. strobus* L., and several of the southern pine species. Coloration resulted from the secretion of a soluble pigment that was taken up by adjacent wood cells, staining them lightly red or purple, depending upon the acidity or alkalinity of the wood. Both Gerry⁸ and Hedgcock⁹ found that certain molds which usually occurred superficially on lumber were capable of shallow penetration and often produced mild interior discoloration.

In so far as could be determined, the fungi associated with the stains herein described, with the exception of one of the surface molds, are distinct from the species mentioned previously. It is probable that a considerable number of fungi are capable of producing less common stains of this nature under favorable conditions, although their distribution may be more or less limited regionally.

EXPERIMENTAL METHODS

A large number of isolations were made in both the field and the laboratory by gouging slivers from the surface and interior of discolored wood and transferring them to malt-agar slants. Those organisms that were isolated repeatedly and seemed involved in the staining of the wood were selected for the purpose of this study. Duplicate agar cultures of the *Fusaria* were sent to Doctor Sherbakoff and duplicate agar cultures of the other organisms to Doctor Thom for identification. To determine their staining ability and characteristics, each organism was planted upon culture blocks of pine and red gum sapwood. Previous to inoculation with the several fungi, the culture blocks had been submerged in boiling water for five minutes and transferred immediately to sterile 2-quart fruit jars plugged with cotton. After an incubation period of 50 days, reisolations were made from the resultant stains, and the gross cultural characteristics of the organisms on agar were compared with those of the original cultures. Field observations were correlated with cultural results wherever possible.

RESULTS

Under the almost ideal conditions for growth presented in the laboratory, all the organisms covered the blocks of both pine (*Pinus palustris* and *P. taeda*) and gum with a superficial growth within 7 to 10 days. Those capable of staining produced definite discoloration in the interior of the blocks within 30 days after inoculation. For convenience, the stains are grouped into four classes: (1) Purple-pink stains, (2) orange stain, (3) yellow stain, and (4) superficial mold stains. The organisms involved and some of their staining characteristics and effects on wood are summarized in Table 1.

⁸ GERRY, E. FIVE MOLDS AND THEIR PENETRATION INTO WOOD. Jour. Agr. Research 26: 219-230, illus.: 1922.

⁹ HEDGCOCK, G. C. Op. cit.

TABLE 1.—Effect of minor staining fungi on wood

Stain and fungus	Wood from which fungus was isolated	Type of stain		Distribution of fungus in wood	Factors producing the stain
		In field	In culture blocks		
Purple-pink stains: <i>Fusarium moniliforme</i>	Sapwood of southern pine; red gum.	Purple and pink patches on pine; faint purple flecks on red gum.	Purple color throughout pine; small purple patches in red gum.	Confined largely to ray parenchyma and resin ducts of pine.	Colored hyphae and secretion of soluble pigment.
<i>Fusarium solani</i>do.	Purple patches, less intense on gum.	Similar to that of <i>F. moniliforme</i> , but less intense.	.do.	Do.
<i>Fusarium rividei</i>	Southern pine	.do.	Similar to that of <i>F. solani</i> on pine; less intense on gum.	Ray parenchyma and resin canals.	Do.
Orange stain: Unidentified fungus.....	.do.	Small shallow orange spots.	Shallow orange spots.	Vigorous surface growth; slight penetration into rays and resin ducts.	Do.
Yellow stain: <i>Gymnoscesuslike fungus</i>	Southern pine; red gum.	Yellow patches on pine; shallow spots on gum.	Yellow coloration throughout pine; shallow spots on gum.	Ray parenchyma, resin canals, to some extent in tracheids. No bore holes evident.	Largely colored hyphae and spores; soluble pigment to some extent.
Superficial stains: <i>Penicillium czpanauum</i>	Southern pine	Bluish-green surface discoloration; often general over piece.	Darkening of wood limited to immediate region of resin ducts.	Resin ducts principally; to some extent ray parenchyma and tracheids.	Compacting of hyphae and fruiting structures in resin ducts.
<i>Glodidium</i> sp. and <i>Trichoderma</i> sp.	Southern pine; red gum	Green surface discoloration; often associated with blue-stain log infection.	.do.	.do.	Do.

The purple-pink stains caused by several species of *Fusarium* are of minor economic importance. They occur in small patches and do not involve the entire sapwood area, as the blue stain often does. They appear on lumber and logs in the early stages of drying and are prevalent only when moisture is unusually abundant. Under favorable conditions of moisture interior discoloration may be produced in lumber within 10 to 15 days after it is sawed. No reliable distinction can be made in the field between the several organisms involved, since the color differences exhibited on wood are slight, even in fresh cultures, and tend to fade as the wood dries. Moreover, differentiation is difficult in the laboratory because the several species vary considerably in color, form, and characteristics of growth, depending on the substratum upon which they are grown. Staining is accomplished through the presence of colored hyphae and the secretion of a soluble pigment which is absorbed by adjacent wood cells. Of the three species, *Fusarium moniliforme* Sheld. was isolated most frequently and produced the most intense discoloration both in the field and in the laboratory. *F. solani* (Mart.) Sacc. was often associated with purplish discolored areas in stored red gum logs, and is possibly the principal cause of such stains. The third species, provisionally identified as *F. viride* (L.) Wr., produced a heavy superficial growth of mycelium on culture blocks, but proved less capable of staining the wood than either of the other organisms.

The orange stain is produced in pine lumber and culture blocks by a fungus which could not be identified because of its failure to fruit. On Difco malt agar the mycelium is cream to buff-orange and semi-appressed; it secretes a pigment that gives the agar a color varying from orange to coffee. This stain was found infrequently in the field and rarely assumed dimensions greater than small shallow spots or patches. It is probable that in many instances it was overlooked because of its inconspicuousness.

Under certain conditions, the yellow stain is common on pine lumber and occurs to some extent on red gum also. This stain appears on lumber in the early stages of drying and is prevalent only when there is an exceptional quantity of moisture present. The yellow color, although bright at first, tends to fade as the wood dries. Numerous isolations from such stained areas yielded only one type of organism, which was associated frequently with *Gliocladium* or *Trichoderma* mold. This organism produces asci abundantly. These show the characteristic shape of certain species of *Aspergillus*, but there are no *Aspergillus* heads on the colony. According to Thom, the general character of the colony is that of *Gymnoascus*, corresponding closely in form to *Penicillium luteum* Zuk. and its allies, but having an entirely different form of ascospore. On Difco malt agar, the mycelium and spores are from the first a decided lemon yellow, and in older cultures the agar may be changed to a definite brown. Hedgcock¹⁰ describes *Penicillium aureum* as creating a lemon-yellow stain in pine wood or in acid media. This organism was not obtained in any of the isolations made in this study.

Superficial mold stains of several different colors occur on moist surfaces of pine lumber and logs and to some extent on gum, within as short a period as 36 to 48 hours after they are sawed. The stains

¹⁰ HEDGCOCK, G. C. Op. cit.

appear first as white superficial growths and later assume their characteristic colorations on the surface of the wood. In the case of pine, discoloration is limited to a darkening of the wood in the immediate region of the resin ducts where the fungus filaments and spores are compacted. Of these molds, *Penicillium expansum* (Lk.) Thom showed a definite preference for both pine and gum lumber that had been treated with organic mercury salts, such as ethyl mercury phosphate and ethyl mercury chloride. *Gliocladium* sp. and *Trichoderma* sp. were encountered wherever lumber was stored under moist conditions. They seemed especially prevalent on lumber cut from infected logs, where they were usually associated with areas of blue stain. Several dipping treatments for lumber, containing fluoride salts, sodium bisulphite, formaldehyde, or arsenious oxide, seemed to encourage the development of molds of this type. The treatments giving the most satisfactory control were those with sodium carbonate and bicarbonate, sodium dinitrophenolate, sodium orthophenyl phenolate, or lime sulphur. The genus *Gliocladium* is regarded by Hedgcock¹¹ as one of minor importance in the staining of lumber.

SUMMARY

A number of stains other than those of the ordinary blue-stain type were found on lumber and logs stored under exceptionally moist conditions. The staining capacity of the fungi that were isolated repeatedly from such discolored wood was determined by inoculating culture blocks of sap pine and gum. Most of them proved to be superficial molds, but several produced stains, including purple-pink, yellow, and orange, throughout the blocks. Most of these stains belonged to the *Fusarium* and *Penicillium* groups. The more common superficial molds were found to produce either uniform shallow discolorations or discolorations limited to the immediate region of the resin ducts. Among the most common genera represented were *Penicillium*, *Trichoderma*, and *Gliocladium*.

These stain fungi were somewhat similar to the blue-stain type in that the mycelium was concentrated in the resin ducts and parenchyma cells of the wood rays but occurred to a limited extent in the wood and ray tracheids. Coloration was due either to the color of the mycelium alone or to the combined color of the mycelium and a soluble pigment that was absorbed by the adjacent wood cells.

Certain chemical solutions with which the lumber had been treated in order to prevent stain appeared to stimulate the growth of some of these organisms.

¹¹ HEDGCOCK, G. C. Op. cit.

THE RELATION OF AGRONOMIC PRACTICE TO THE QUANTITY AND QUALITY OF THE OIL IN FLAX-SEED¹

By I. J. JOHNSON²

*Assistant Agronomist, Division of Agronomy and Plant Genetics, Minnesota
Agricultural Experiment Station*

INTRODUCTION

Present-day problems in agronomy are as much concerned with factors that affect the quality as with those that affect the quantity of the various crop plants. It is not only essential carefully to survey existing varieties in order to determine the range of their variability in composition, but it is also of paramount importance to be able to predict the effect of different agronomic practices on the quality of the crop. Such knowledge is available for many of the commonly grown crop plants, but for flax information of this sort is rather limited. Since agronomic practices in flax production are more variable than for many other crops, it is desirable to know in greater detail the relation between cultural practices and the composition of the seed.

The present series of investigation with flax were made to obtain additional data on the rate of development of the seed and the formation of oil on soils of different productivity, and to study the influence of delayed planting on the yield and composition of the seed.

PREVIOUS INVESTIGATIONS

Several investigators have studied the influence of fertilizer applications on the oil content of oil-bearing seeds. The studies with flax (6, 7, 11)³ have shown that applications of commercial fertilizers may alter the oil content of the seed and also influence the various constituents of the extracted oil (11). Garner, Allard, and Foubert (8) have shown that the oil content of cottonseed from plots where complete fertilizers were used was greater than that of the seed from the check plot, but that heavy applications of nitrogen relative to phosphorus and potassium reduced the oil content of the seed. Stark (13) determined the oil content of soybeans under various soil treatments and found that applications of lime and organic matter tended to reduce the oil content of the seed, and that rock phosphates added with lime and organic matter still further reduced it. Potash applications increased the oil content of the seed.

¹ Received for publication Jan. 19, 1932; issued September, 1932. Paper No. 1075, of the Journal Series, Minnesota Agricultural Experiment Station. This paper formed part of a thesis submitted to the faculty of the University of Minnesota in partial fulfillment of the requirements for the degree of doctor of philosophy, granted June 8, 1931.

² The author wishes to express his appreciation to Prof. A. C. Arny for suggestions in the planning and conduct of these experiments; to Dr. H. K. Hayes, Chief of the Division of Agronomy and Plant Genetics, for suggestions in the presentation and summarization of the experimental results; to the Division of Agricultural Biochemistry, University of Minnesota, for the use of laboratory apparatus; and to the Archer-Daniels-Midland Co., Minneapolis, Minn., for determinations of the percentage of oil and iodine number on the seed from the dates of planting.

³ Reference is made by number (italic) to Literature Cited, p. 255.

Bushey, Puhr, and Hume (3) have studied the influence of crop sequence on the oil content of flaxseed. They analyzed 10 samples representing 10 rotations and found a difference of only 2.1 per cent between the sample containing the least oil and that containing the most. The differences obtained were not considered to be significant.

Rabak (12) has made an extensive study to determine the influence of climate and soil on the percentage and quality of oil in flaxseed. From four varieties of flax grown in 1914 and 1915 at seven locations in the middle and far-western part of the United States a considerable variation in the oil content of the seed was obtained. Tobler (14) has summarized the work of Ivanow, who studied the percentage and iodine number of the oil from flaxseed secured from 28 experiment stations in the flax-producing countries of the world. The oil content of the seed was found to be dependent on the kind (variety) and was not strongly influenced by geographical location. The iodine number of the oil, however, was influenced by the region in which the seed was grown. Seed secured from northern latitudes gave a higher iodine number than seed produced in southern countries.

A summary of the data obtained by the investigators referred to indicates that flaxseed may, under certain conditions, show considerable variability in composition as a result of differences in soil fertility and climate. Only a relatively small number of investigations have been made on the various phases of oil formation in oil-bearing seeds. Most of the studies with flaxseed have been made with bulk samples secured by harvesting flax plants at various stages in their development. Since flax blooms over a period of two weeks or more, bolls of different ages are found on the same plant. The difficulty of determining the exact stage of development of the seed by this method of harvesting is quite evident. Ivanow (10) harvested flax at four stages of maturity to study the oil-forming process. In 1910 the seed from the first harvest one week after flowering contained 4.4 per cent oil, the seed from the second harvest on July 18, 13 days later, contained 11 per cent, the seed from the third harvest on August 3 contained 32.5 per cent, and the seed from the fourth harvest on August 25 contained 35 per cent. These data show an extremely rapid rate of oil formation between July 18 and August 3, when practically all of the oil was laid down in the seed. The seed secured from each harvest was analyzed to determine the percentage of sugars. As the seed matured the percentage of sugars diminished, which suggests their conversion to oils during the oil-forming process. From a study of the percentage of oleic and linoleic acids and the iodine number of the oil at each harvest, Ivanow concluded that the saturated fatty acids were formed first during the synthesis of oil and from them the unsaturated fatty acids were derived.

The analysis of flaxseed for oil content at four degrees of maturity made by Eyre and Fisher (6) gave results which agreed quite closely with those of Ivanow (10). Eyre and Fisher suggest that flax may be harvested before it is fully mature without a serious reduction in the yield of oil. This is a common practice where flax is grown as a fiber crop.

Bushey, Puhr, and Hume (3) harvested flax planted on June 10 at five stages of maturity. The seed from the first harvest on August 25 was very immature but contained 29.51 per cent oil. The seed from

the fourth harvest on September 21, which was fully matured, contained 36.84 per cent. The iodine number of the extracted oil was lower at each of the five harvest dates. The maximum value was 181.5 at the first harvest and the minimum was 161.0 at the last harvest.

Dillman (5) has measured the growth in volume and the formation of oil in developing flaxseed. The seed used for the study was of known age, obtained by tagging a large number of flowers and collecting the seed at frequent periods of their development. It is evident that a study made by this method gives more accurately the rate of development of the seed, since the material used for each of the harvests is of uniform and known age. The results of Dillman's studies made in 1926 and 1927 at University Farm, St. Paul, Minn., and at Mandan, N. Dak., show that the growth in volume of the seed, as determined by measurements of length, width, and thickness, is relatively rapid, reaching a maximum at 12 to 14 days after flowering. The growth of the seed, as determined by the daily increase in dry weight, continued for a period of 33 days and then remained constant to the end of the ripening period at 40 days after flowering. The most rapid formation of oil, based on percentage of oil in the dry seeds, began at about the seventh day after flowering and continued for a period of 15 to 18 days. After the maximum percentage of oil was reached there was little or no significant change up to full maturity. The maximum percentage of oil was reached somewhat before the maximum dry weight of the seed.

All the data reviewed indicate that oil formation in oil-bearing seeds takes place most rapidly midway between flowering and maturity. Consequently, a study made during this period should show most clearly the relation of environmental conditions to oil content of the seed.

Bushey, Puhr, and Hume (3), working with flax, and Garner, Allard, and Foubert (8), with soybeans, have shown that delayed planting has very little effect upon the oil content of the harvested seed. In each instance, plantings were made at 15-day intervals over a 2-month period beginning at the normal planting date for the crop used. Each of these studies was conducted for a single year. One variety of flax and four varieties of soybeans were included in the tests. Stoa⁴ has furnished unpublished data on the oil content of flaxseed from several dates of planting. His studies, covering a 7-year period, show conclusively that delayed planting has only a very slight influence on the oil content of the seed.

MATERIALS AND METHODS

Two varieties of flax, Bison (Minnesota accession No. 199) and Diadem (Minnesota accession No. 197) were used to determine the relation of soil productivity to the rate of seed and boll development and oil formation. These varieties were selected on the basis of considerable agronomic data from varietal trials at University Farm, Minn., and at branch stations. Bison is characterized by a fairly tall growth habit, mid-season maturity, and medium large seeds, and it has yielded well in variety trial comparisons. Diadem is mid short in growth habit, later in maturity, has fairly large seeds, and has given

⁴ Stoa, T. E. Unpublished data.

comparatively low yields in varietal trials. The two varieties are therefore quite different in agronomic characteristics.

The two varieties were grown in 1929 on plots represented by two different rotations and a continuous cropping system. The plot treatments were started in 1899 and have been in continuous operation since. The 5-year rotation is composed of wheat, clover and timothy hay, pasture, corn, and oats. Barnyard manure has been applied preceding the corn crop. The 3-year rotation includes wheat, clover and timothy, and corn. In the continuous wheat, 6 pounds of red clover was planted each year and the crop growth was plowed under in the fall. No manure or fertilizer has been applied to the crops on the 3-year rotation or to the continuous wheat. The rotation plots were arranged to grow each crop every year, so that yield comparisons are available for all three plots used throughout the entire period of cropping. The yields of wheat in bushels per acre for the 4-year period 1924-1927 are 22.6 for the 5-year rotation, 18.6 for the 3-year rotation, and 15.3 for the wheat continuous. These data show considerable difference in the yield of wheat under the three different cropping systems.

The two varieties were planted on May 1 with a field grain drill on a portion of each of the three plots usually planted to wheat. Shortly before full bloom enough flowers were marked to supply seed for daily measurements and oil determinations. The flowers were marked by tying with a loose knot a small piece of colored woolen yarn just below the flower. Since it was impossible to tag on the same day all the flowers needed from each of the plots, a different colored yarn was used for each tagging date. Twelve thousand to thirteen thousand flowers were marked on each variety for each of the three plots, making a total of 75,000 flowers used in the experiment.

At 7 o'clock every morning from the date of flowering to maturity 40 bolls of each variety were collected from the three plots and taken to the laboratory for analysis. As the bolls were gathered they were put in small tightly stoppered glass jars to minimize the moisture loss. The length and diameter of 10 bolls were measured with a vernier caliper, and the length, width, and thickness of five seeds from each of five bolls were measured with a dissecting binocular fitted with a micrometer. All measurements were read to 0.1 mm. To facilitate measuring, the five seeds from each boll were placed on a glass slide, and after the length and width had been recorded each was turned on edge with a needle and the thickness measured near the center of the seed. Duplicate 100-seed samples were removed from the remaining bolls and dried to constant weight in an electric oven at 70° C.

During the afternoon of each day, from five days after flowering until maturity, enough bolls were collected from each plot to provide material for oil determinations.

The harvested bolls were placed on trays with screened bottoms to dry in the open air during the day. At night the trays were taken into the laboratory and the drying was continued in a current of air supplied by electric fans. When the bolls were well dried they were placed in thin cloth bags and hung in a well-aired room until threshed.

To determine the relation of planting date to yield and composition of the seed, four varieties of flax were planted at University Farm in 1929 and 1930 at successive 10-day intervals, beginning on May 1.

The four varieties used were Redwing (Minnesota accession No. 188), Bison (Minnesota accession No. 199), Buda (Minnesota accession No. 194), and Winona (Minnesota accession No. 182). The first two are the recommended varieties for the State. These varieties were grown in 3-row triplicated rod-row plots in 1929 and in 5-row triplicated plots in 1930. The center row was used in 1929 for yield and other plant characters and for study of the composition of the seed. The second row in the 5-row plots was harvested in 1930 for yield, and the fourth row was used for the study of plant characters.

The methods outlined by the Association of Official Agricultural Chemists (2) were used in the determination of the percentage and iodine number of the oil. The seed was finely ground in a special mill adapted to flaxseed, described in detail by Coleman and Fellows (4). Duplicate 1-gram samples were used in the study of the daily increase in oil content. The extracted oil was dried in an electric oven at 80° C. for one and one-half hours, then weighed and redried for an additional half hour. The loss in weight during the final half hour was usually slight. Iodine numbers were determined on the oil secured from the ether extraction. The weighed oil was dissolved in 20 c c of chloroform and transferred to ground-glass stoppered flasks used for iodine absorption reaction. Wijis solution was used in all determinations of the iodine absorption number.

EXPERIMENTAL RESULTS

THE DAILY INCREASE IN SEED SIZE, BOLL SIZE, AND OIL CONTENT OF FLAX ON SOILS OF DIFFERENT PRODUCTIVITY

Daily measurements of the length, width, and thickness of the seed and the length and diameter of the boll were made with two varieties during the entire growth period from flowering to maturity. These measurements for flax grown on the different rotations show only a slight relation to soil productivity. The greatest difference occurred during the early part of the growth period, when the seed and boll dimensions were increasing most rapidly. After the approximate maximum size had been reached there was no consistent difference between the measurements of the bolls or seeds produced on the three plots. The daily measurements for the first seven days after flowering and the average of successive 7-day periods to maturity are given for both of the varieties in Table 1.

TABLE 1.—*Relation of soil productivity to daily and periodical increase in seed length, width, and thickness, and to boll length and width (millimeters), of Bison and Diadem flax grown on a 5 and 3 year rotation and a continuous cropping system*

Days after flowering	Measurements of Bison grown in—			Measurements of Diadem grown in—		
	5-year rotation	3-year rotation	Continuous cropping	5-year rotation	3-year rotation	Continuous cropping
0.....	1.16	1.21	1.00	1.30	1.34	1.36
1.....	1.45	1.44	1.44	1.64	1.70	1.69
2.....	2.14	2.08	2.26	2.08	2.17	2.20
3.....	2.50	2.67	2.68	2.62	2.71	2.89
4.....	3.25	3.30	3.53	3.27	3.29	3.37
5.....	4.03	3.31	4.17	3.25	3.39	3.56
6.....	4.38	4.04	4.65	4.02	4.09	4.28
7.....	4.72	4.45	4.79	4.45	4.43	4.38
8-14.....	5.10	5.09	5.03	5.24	5.10	5.12
15-21.....	5.12	5.16	5.12	5.27	5.37	5.26
22-28.....	5.25	5.23	5.25	5.31	5.32	5.38
29.....	5.15	5.04	4.97	5.26	5.24	5.28

SEED WIDTH						
0.....	0.55	0.60	0.50	0.66	0.70	0.69
1.....	.75	.74	.74	.80	.91	.88
2.....	1.10	1.12	1.16	1.13	1.19	1.18
3.....	1.31	1.39	1.49	1.43	1.48	1.50
4.....	1.72	1.82	2.05	1.82	1.90	1.85
5.....	2.14	1.79	2.29	1.75	1.87	1.95
6.....	2.44	2.24	2.70	2.21	2.31	2.32
7.....	2.76	2.45	2.86	2.54	2.45	2.54
8-14.....	2.94	2.97	2.94	3.01	2.96	2.99
15-21.....	2.97	2.99	2.92	3.07	3.01	3.03
22-28.....	3.01	3.01	2.95	3.00	3.01	3.05
29.....	2.88	2.72	2.65	2.80	2.79	2.89

SEED THICKNESS						
0.....	0.38	0.37	0.34	0.44	0.40	0.44
1.....	.50	.50	.48	.52	.59	.54
2.....	.66	.62	.68	.68	.68	.66
3.....	.77	.78	.80	.80	.80	.86
4.....	.92	.91	1.10	.94	.93	.95
5.....	1.11	.98	1.02	.98	1.00	1.02
6.....	1.26	1.17	1.38	1.15	1.14	1.16
7.....	1.45	1.23	1.48	1.23	1.24	1.26
8-14.....	1.54	1.50	1.47	1.46	1.43	1.41
15-21.....	1.51	1.47	1.42	1.47	1.47	1.42
22-28.....	1.57	1.48	1.41	1.54	1.52	1.49
29.....	1.45	1.24	1.14	1.43	1.39	1.38

BOLL LENGTH						
0.....	3.35	3.48	3.16	4.05	4.14	4.32
1.....	3.98	3.87	3.51	4.18	4.31	4.55
2.....	4.59	4.48	4.20	4.97	5.28	4.80
3.....	5.03	4.98	4.92	5.33	5.54	5.69
4.....	5.60	5.88	5.66	6.03	6.34	6.48
5.....	6.02	5.50	5.89	5.59	5.77	6.02
6.....	6.59	6.20	6.76	6.50	6.50	6.77
7.....	7.00	6.68	6.85	6.76	7.00	6.94
8-14.....	7.52	7.65	7.39	7.99	7.97	8.18
15-21.....	7.64	7.73	7.57	8.10	8.54	8.41
22-28.....	7.69	7.77	7.62	8.24	8.44	8.42
29.....	7.54	7.66	7.51	8.50	8.57	8.31

BOLL WIDTH						
0.....	2.39	2.45	2.10	2.59	2.56	2.68
1.....	2.78	2.87	2.68	3.10	3.16	3.17
2.....	3.64	3.52	3.52	4.01	4.13	4.03
3.....	4.50	4.60	4.48	4.58	4.63	4.75
4.....	5.21	5.30	5.17	4.60	5.40	5.30
5.....	5.48	5.38	5.58	5.11	5.05	5.30
6.....	5.61	5.62	6.36	5.61	5.79	5.88
7.....	6.42	5.95	6.62	5.88	6.08	6.06
8-14.....	6.96	7.02	6.93	6.96	6.83	6.99
15-21.....	7.16	7.20	7.00	7.03	7.19	7.22
22-28.....	7.14	7.25	7.07	7.07	7.11	7.37
29.....	7.16	7.18	7.05	7.11	7.03	7.21

Statistical comparisons of the daily boll and seed measurements have been made for the first 7-day period after flowering. By the use of Student's pairing method (1) and table of Z (9) the odds against the chance occurrence of differences as large or larger than those found have been determined. The mean differences and odds are summarized in Table 2.

TABLE 2.—*Statistical comparison of the mean difference in seed and boll size (millimeters) of two flax varieties, grown on soils of different productivity, with odds that differences are significant*

Measurement	Variety	5 and 3 year rotation		5-year rotation and continuous cropping		3-year rotation and continuous cropping	
		Mean difference	Odds	Mean difference	Odds	Mean difference	Odds
Seed length.....	Bison.....	0.0714	5:1	-0.1625	21:1	-0.2371	22:1
	Diadem.....	-.0738	196:1	-.1375	151:1	-.0703	36:1
Seed width.....	Bison.....	.0775	6:1	-.1225	70:1	-.1750	40:1
	Diadem.....	-.0588	47:1	-.0788	184:1	-.0200	46:1
Seed thickness.....	Bison.....	.0652	27:1	-.0538	22:1	-.1150	66:1
	Diadem.....	-.0038	1:1	-.0188	27:1	-.0150	6:1
Boll length.....	Bison.....	.1363	9:1	.1388	25:1	.0150	1:1
	Diadem.....	-.1838	1000:1	-.2700	302:1	-.0803	5:1
Boll diameter.....	Bison.....	.0050	1:1	-.0000	2:1	-.0050	2:1
	Diadem.....	-.0425	5:1	-.0088	20:1	-.0503	6:1

From the data given in Table 2 it is apparent that no consistent difference exists between the measurements of the bolls and seeds produced on the 3-year and 5-year rotation plots. Although the length and width of the seed and the length of the boll are significantly smaller for Diadem grown on the 5-year rotation plot, the differences (which are small) are in the opposite direction for Bison. This suggests a varietal response to soil productivity. A comparison of the seed and boll measurements from the 5-year rotation with the continuous cropping plot shows, with the exception of the boll length and diameter of Bison, a significant difference in favor of the continuously cropped plot. The comparisons of the boll and seed measurements from the 3-year rotation and the continuous cropping plot indicate a somewhat larger seed and boll size from the latter plot. The odds are not, however, as large as between the boll and seed measurements from the 5-year rotation and the continuous cropping plot.

The relation of soil productivity to the daily increase in dry weight of the seed is shown in Table 3.

TABLE 3.—*Relation of soil productivity to the average daily increase in dry weight (grams) of 100 seeds of Bison and Diadem flax grown in a 5 and 3 year rotation, and a continuous cropping system*

Days from flowering	Weight of 100 seeds from Bison grown in—			Weight of 100 seeds from Diadem grown in—		
	5-year rotation	3-year rotation	Continuous cropping	5-year rotation	3-year rotation	Continuous cropping
0.	0.0080		0.0076	0.0104		0.0100
1.	.0104	0.0108		.0144	0.0120	.0124
2.	.0131	.0129	.0152	.0192	.0134	.0146
3.	.0203	.0202	.0233	.0202	.0172	.0240
4.	.0382	.0383	.0471	.0318	.0295	.0421
5.	.0612	.0416	.0794	.0337	.0350	.0436
6.	.0792	.0653	.1088	.0591	.0508	.0692
7.	.1037	.0688	.1224	.0668	.0801	.0858
8.	.1572	.1169	.1399	.1199	.0994	.1237
9.		.1386	.1315	.1078	.1133	.1407
10.	.1572	.1614	.1633	.1065	.1700	.1866
11.	.1832	.1858	.2145	.1804	.1858	.1974
12.	.2027	.1837	.2606	.1983	.2267	.2375
13.	.2331	.2307	.2677	.2774		
14.	.2357	.2475	.2872	.2681	.2634	.2610
15.	.2575	.2693	.3108	.2515	.2428	.2836
16.	.2960	.2960	.3492	.2798	.2989	.3087
17.	.3022	.3060	.3834	.3157	.3207	.3182
18.	.3459	.3756	.4262	.3200	.3716	
19.	.4342	.4282	.5049	.3222	.3085	.3128
20.		.4452	.5321	.3410	.4376	.3198
21.	.4820	.4813	.5543	.3618	.4422	.3992
22.	.5010	.5069	.5675	.4157	.4925	.4741
23.	.5245	.5288	.5677	.4342	.4847	.4444
24.	.5773	.5311	.5472	.5632	.5521	.5427
25.	.5870	.5394	.5985	.5616		.5437
26.	.5889	.5432		.5820		.5565
27.	.5925	.5730	.5806	.6438	.6110	.5713
28.	.6046	.5709		.6566	.5918	.5542
29.	.6022	.5801	.5854	.6997	.6965	.6411
30.	.5994	.6062		.6838	.6794	.6466
31.	.5996	.6258	.5758	.6814	.6866	.6543
32.	.5998	.6023		.7019	.7019	
33.	.6518	.5809	.5790	.6657	.7150	.6284
34.	.6516	.6188		.6780	.6720	.6281
35.	.6392			.7025	.6992	.6438
36.	.6581			.6622	.7092	.6356
37.	.6760			.7296		
38.				.7286		
39.				.7018		

TABLE 4.—*Statistical comparisons of the mean difference in daily increase in dry weight (grams) of seed from soils of different productivity with odds that differences are significant*

Period of pro- ductivity	Variety	5 and 3 year rotation		5-year rotation and continuous cropping		3-year rotation and continuous cropping	
		Mean dif- ference	Odds	Mean dif- ference	Odds	Mean dif- ference	Odds
First	{Bison	0.007114	14:1	—0.021250	450:1	—0.027914	2,700:1
	{Diadem	—0.006356	1:1	—0.012073	324:1	—0.012700	1,374:1
Second	{Bison	—0.021150	1:1	—0.055663	470:1	—0.061045	5,000:1
	{Diadem	—0.028873	22:1	0.011000	4:1	0.035460	243:1
Third	{Bison	—0.023780	30:1	0.024760	15:1	0.040020	1:1
	{Diadem	—0.00325	5:1	0.042200	10,000:1	0.028857	4,000:1

Comparisons of seed weight of the flax grown in the three systems of cropping have been made for three periods of growth. The first period includes the first 15 days after flowering, the second from 15 days until the maximum seed size was reached, and the third from

maximum seed size to maturity. The approximate maximum seed size was reached on the twenty-fourth day after flowering for Bison and on the twenty-eighth day for Diadem. The mean difference and the odds derived from student's pairing method and tables of Z are given in Table 4.

The results obtained indicate approximately the same response to soil productivity as was shown by the seed and boll measurements. (Table 1.) The differences in seed weight of flax produced on the 3 and 5 year rotation plots are not significant, but the odds are great that during the first two growth periods the seed weight obtained from either of the rotation plots is less than that obtained from the continuous cropping plot, indicating that the seed developed more rapidly on the soil of lowest productivity. During the final growth period when the increase in seed weight was slight, the above relation was reversed for Diadem only, and the odds are great that the seed weight is less from the continuous cropping system than from either of the rotations. The difference in varietal response during the final growth period suggests that Bison is better suited to soil of low productivity than Diadem.

Determinations of the daily increase in oil content were begun at five days after flowering for the 3-year rotation and at seven days after flowering for the 5-year rotation and the continuous cropping system. At the 5-day stage there was considerable difficulty in separating the very small seeds from the threshed bolls. The percentage of oil from Bison only is given in Table 5. These results, secured at frequent periods of development, show no consistent differences in response to soil productivity for the different cropping systems during the period of oil formation. Statistical comparisons of the oil content made from the twenty-third day to maturity between the flax produced on the 5 and 3 year rotation and the 5-year rotation and continuous cropping plots give a mean difference in favor of the 5-year rotation of 0.56 per cent and 0.87 per cent oil, respectively, with odds of 6:1 and 21:1 that these differences are significant. The comparison between the oil content of the seed from the 3-year rotation and continuous cropping plots gives a mean difference in favor of the former of 0.42 per cent oil. The odds are only 4:1 against the occurrence of differences as large or larger due to errors of random sampling.

TABLE 5.—Relation of soil productivity to the daily increase in percentage oil content of Bison flax based on the dry weight of the seed

Days from flowering	Percentage oil content of flaxseed			Days from flowering	Percentage oil content of flaxseed		
	5-year rotation	3-year rotation	Continuous cropping		5-year rotation	3-year rotation	Continuous cropping
5.....		9.7		19.....	36.0	35.7	37.7
7.....	14.3	13.3	10.3	21.....	37.5	37.7	37.7
8.....		18.2	15.0	23.....	38.0	39.1	39.1
9.....	14.3	18.8		25.....	40.5	39.5	38.7
10.....			21.4	27.....	39.9	39.1	38.4
11.....	34.5	19.0		30.....	39.7	39.1	38.8
13.....	26.6	23.6	31.5	33.....	39.7	38.2	38.9
15.....	29.1	32.1	35.8	35.....	39.3		38.0
17.....	35.1	33.8	36.0				

Varietal differences in the rate of development of seed and boll size, dry weight, and oil content of the seed are shown by the average results from the three cropping systems. Such an average is justifiable from the statistical comparisons previously made. This average also shows more accurately daily changes in size and composition of the seed throughout the entire growth period. The growth curves (fig. 1) from the averaged results show that the length, width, and thickness of the seed increased rapidly, reaching an approximate maximum at nine days after flowering. From this stage until shortly before ma-

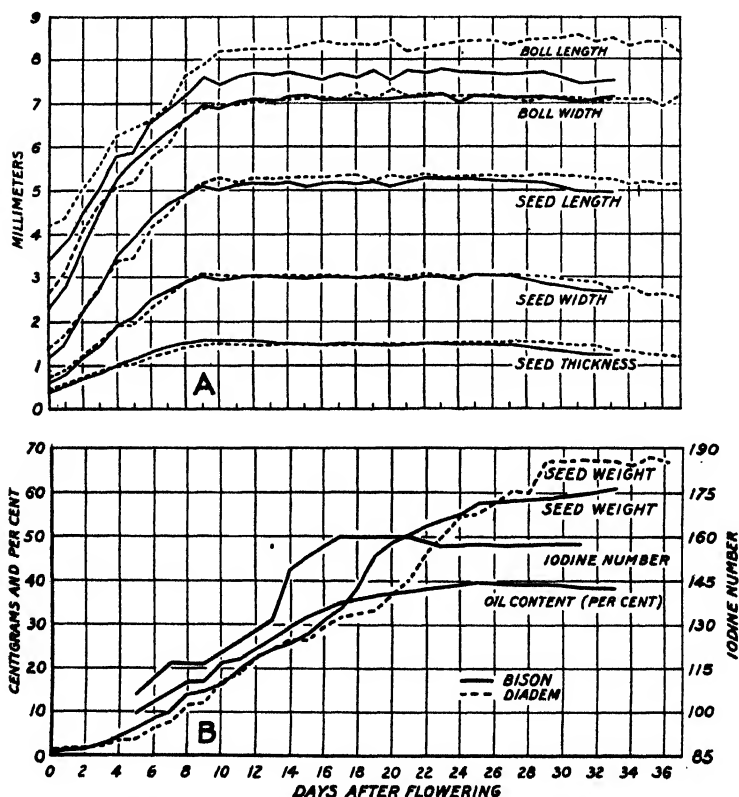


FIGURE 1.—Average daily seed and boll measurements, (A) dry weight of 100 seeds, and the percentage and iodine number (B) of the oil of Bison and Diadem flax

turity there was no great change in seed size. Coincident with the decrease in moisture content with approaching maturity, the measurements show a slight but consistent decrease. Throughout the entire growth period there was a remarkably close agreement in the daily development of the two varieties, which probably indicates that the flax varieties used are quite similar physiologically although different morphologically. The close agreement of these results with those secured by Dillman (5) with the variety Rio (C. I. 280) gives further evidence for this conclusion. Since the maximum seed size is reached

at an early stage of development, it is quite likely that environmental influences have only small opportunity to become manifest.

The daily increase in length and diameter of the boll follows very closely that of seed size, reaching a maximum for both varieties at nine days after flowering. From this stage until shortly before maturity there is no appreciable change in size. The lowering of the moisture content with maturity resulted in a slight reduction in boll size.

The daily increase in dry weight of the seed progresses at a fairly uniform rate until approximately 24 days after flowering for Bison and 28 days after flowering for Diadem. This difference of four days represents the difference in number of days from flowering to maturity of the two varieties, the variety Diadem maturing somewhat later than Bison. After the seeds reached their maximum weight there was no great increase to maturity. These results are again in close agreement with those secured by Dillman (5).

The average oil content shows a fairly uniform increase from 5 to 25 days after flowering, followed by a slight but consistent decrease until maturity. The initial oil content is much higher than was found by Dillman (5). This difference may be explained in part by the difference in analytical method used. Dillman determined the oil content by the refractive index method developed by Coleman and Fellows (4). The accuracy of this method is dependent on a constant initial refractive index of the oil. Since the increase in oil content is positively correlated with an increase in refractive index, it is apparent that an initial refractive index lower than the accepted standard will give a lower oil content than that which actually exists in a given sample. Unpublished data furnished by Anderson⁵ show a high positive correlation between the iodine value and the refractive index. Since the iodine value at early stages of development is much lower than at later stages, it is quite likely that the refractive index of the oil is likewise lower than the standard value. Oil determination by such methods may therefore give erroneous results in this type of study. The results secured during the later stages of development agree very closely with those reported by other investigators.

Determination of the iodine absorption number of the oil was made on seed of Bison obtained from the 3-year rotation plot. The results given in Figure 1 and Table 6 suggest that the fatty acids which make up the oil at early stages of development are more saturated than those at later stages. The progressive change in degree of saturation is completed at 17 days after flowering and remains constant for a short time, then decreases from this point to maturity.

TABLE 6.—*Iodine absorption numbers of Bison flax from the 3-year rotation system on various days after flowering*

Days after flowering	Iodine No.	Days after flowering	Iodine No.	Days after flowering	Iodine No.	Days after flowering	Iodine No.
5.....	106	13.....	131	19.....	160	27.....	157
7.....	116	14.....	148	21.....	160	30.....	158
9.....	116	15.....	153	23.....	158	33.....	158
11.....	124	17.....	160	25.....	158		

⁵ Anderson, R. E., chemist, Archer-Daniels-Midland Co.

These results confirm the conclusion reached by Ivanow (10), and indicate that in the synthesis of the oils in flaxseed the saturated fatty acids are formed first. These are then progressively transformed to acids possessing a greater degree of unsaturation. The view that fatty acids are synthesized from glucose is generally accepted at the present time, although the exact intermediate products are not known. During the ether extraction of seed at the period of five to eight days after flowering, traces of resinous substances were isolated from the extracted oil. Although these substances were originally ether soluble, it was impossible to redissolve them in ether after the ether had been evaporated. Preliminary studies indicate that the seed contained from 0.3 to 0.7 per cent of this insoluble fraction. It is quite possible that these substances may be intermediate condensation products in the synthesis of fatty acids from glucose. No traces of this product were found after the 9-day stage.

THE RELATION OF DELAYED PLANTING TO YIELD AND COMPOSITION OF THE SEED

Extensive investigations at University Farm, St. Paul, with several standard varieties have shown that delayed planting of flax generally results in a considerable reduction in yield of seed. Similar results have been secured by investigators at other experiment stations. The reason for such reductions in yield has not always been apparent, since in most instances the difference in percentage of full stand at each planting date has not been sufficient to bring about wide yield differences. During 1929 and 1930 detailed studies were made to determine whether the reduced yield could be explained on the basis of the average number of seeds per boll and the average number of bolls per plant. The determinations were made on four flax varieties planted in replicated rod-row plots at five successive 10-day intervals beginning on May 1. In 1930 an attempt was made to establish a uniform stand at each planting date by thinning the plants when about 1 foot tall to a distance of 1 inch apart within the row. This distance permitted thinning for the first three dates, but a poor stand gave for the last two plantings an average spacing greater than for the first three. In 1929 the plants were not thinned. The stand, however, was approximately uniform for all dates.

The average number of bolls per plant was determined from counts of approximately 400 plants uniformly distributed among three replicates. Between 400 and 500 bolls selected at random from the plants were threshed individually to determine the average number of seeds per boll.

The results secured from each of the varieties for five dates of planting show a remarkably close agreement in 1929 between the decrease in yield and the reduction in number of seeds per boll. (Table 7.) Of the four varieties, Redwing and Winona show the least reduction in yield and in seeds per boll as a result of delayed planting. Although the average number of bolls per plant was greatly decreased by delayed planting, its relation to yield is not as striking as is the number of seeds per boll.

TABLE 7.—*Relation of delayed planting to the yield of seed, average number of seeds per boll, and average number of bolls per plant of four flax varieties planted at successive 10-day intervals*

[The yield data are given in percentage of that obtained from the May 1 planting]

SEED YIELD IN PERCENTAGE

Variety	Planting date, 1929					Planting date, 1930				
	May 1	May 10	May 20	June 1	June 10	May 1	May 10	May 20	June 1	June 10
Redwing.....	100	99.5	77.3	19.3	3.9	100	78.8	58.0	35.9	13.9
Bison.....	100	72.2	66.1	8.7	2.6	100	69.3	38.0	20.0	4.4
Buda.....	100	46.2	41.8	6.6	2.2	100	64.9	33.6	16.0	5.3
Winona.....	100	80.3	73.2	23.6	10.2	100	92.3	63.2	51.6	21.3
Weighted average.....	100	80.0	68.1	16.3	5.2	100	76.2	49.2	21.0	11.0

NUMBER OF SEEDS PER BOLL

Redwing.....	7.80	7.79	7.10	3.28	0.83	8.31	8.77	7.63	5.98	5.54
Bison.....	6.24	6.00	3.92	.65	.43	7.29	6.44	4.49	2.91	4.90
Buda.....	6.82	5.66	4.99	1.07	.27	6.74	4.42	4.37	3.20	4.18
Winona.....	7.95	7.37	6.89	3.00	1.28	7.49	7.40	6.88	5.37	5.20
Average.....	7.20	6.71	5.73	2.00	.70	7.46	6.76	5.84	4.37	4.96
Per cent.....	100	93.2	79.6	27.8	9.7	100	90.6	78.3	58.6	66.5

NUMBER OF BOLLS PER PLANT

Redwing.....	11.22	11.47	7.16	6.97	5.69	15.52	15.86	9.73	8.92	10.84
Bison.....	15.93	14.27	10.63	7.13	5.75	20.36	15.94	10.24	10.15	4.48
Buda.....	15.00	18.32	10.53	7.28	4.18	16.86	17.29	9.90	10.98	4.84
Winona.....	17.28	15.02	11.36	8.57	9.07	19.02	15.76	9.02	14.48	10.89
Average.....	14.86	14.77	9.92	7.49	6.17	17.94	16.21	9.72	11.13	7.76
Per cent.....	100	99.4	66.8	50.4	41.5	100	90.4	54.2	62.0	43.3

In 1930 the relation between yield at the different planting dates and the number of seeds per boll and bolls per plant is not as close as in the previous year. This is true especially for the last two dates, where the poor stand probably influenced the results. The four varieties responded in nearly the same way as in the previous year's study, Redwing and Winona being less influenced by delayed planting than Bison or Buda. The average results for the four varieties given in percentage of the yield obtained from the May 1 date of planting are shown for each of the two years in Figure 2.

Although the two years' study has shown a marked reduction in the number of seeds per boll as a result of delayed planting, the exact reason for this response is not known. Pollen sterility resulting from high temperature at flowering time is not a satisfactory explanation, since rudimentary seeds were found to be present in the mature bolls, indicating that fertilization had taken place. It is quite possible that desiccation resulting from high temperatures during the early period of seed formation may have injured the developing ovules. The very immature seeds have a high moisture content (80-83 per cent during the first 12 days in 1929), and moisture loss through the thin wall of the inclosing boll may have reduced their water content to a point where further development could not take place.

The influence of delayed planting on the oil content of the seed was determined from four varieties in 1929 and two varieties in 1930. The iodine number of the extracted oil was made on the 1930 crop. Table 8 shows that delayed planting resulted in a very slight reduction in oil content in 1929, the reduction in Bison being somewhat greater than in the other varieties. In 1930, however, there was no consistent change in either of the two varieties used. These results are in close agreement with those reported by other investigators (3, 9).

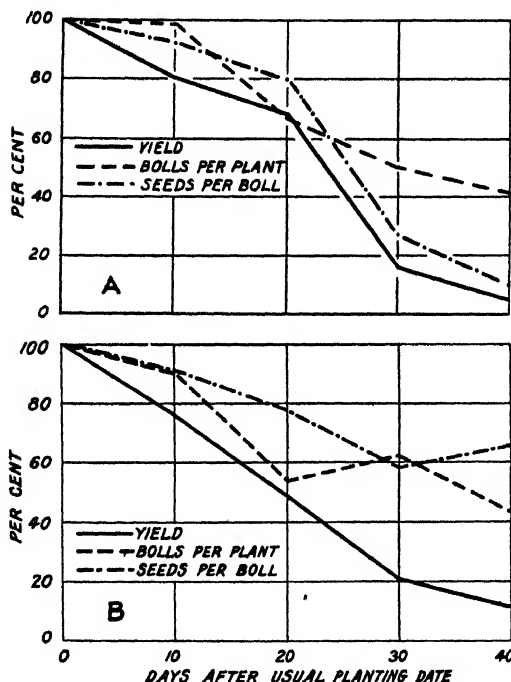


FIGURE 2.—The relation of delayed planting to the yield of seed, number of bolls per plant, and number of seeds per boll in 1929 (A) and 1930 (B). The data are given in percentages of those obtained from the May 1, or usual, planting date

by Bushey, Puhr, and Hume (3) likewise show a lowering of the iodine number with progressive maturity. These results suggest that the high iodine number of the seed from the last date of planting was due to its immaturity.

The results of these investigations, together with those previously reported, show conclusively that oil content is less influenced by environmental changes than are the other plant characters studied. The maintenance of uniformity in oil content while other characters were changed by delayed planting suggests that the composition of flaxseed is a fairly constant heritable character. The uniformity in oil content does not, however, justify late planting of flax, inasmuch as this practice results in a lower yield of seed.

The data from the study of the iodine number of the oil from the 1930 crop show a consistent reduction for Bison for the first four dates but no significant change for Redwing. Both varieties had a higher iodine number for the last date of planting than for the other dates. The seed from this planting was not fully mature when harvested, and therefore the results from this date of planting are not comparable with the others. The studies made on the oil from seed at frequent stages of development gave a somewhat lower iodine number for the fully mature seed than for immature seed at 17 days after flowering. The results secured

TABLE 8.—*Relation of delayed planting to the percentage of oil, iodine number, and average number of days from full bloom to maturity of flax varieties planted at successive 10-day intervals after May 1*

Variety	PERCENTAGE OIL									
	Planting date, 1929					Planting date, 1930				
	May 1	May 10	May 20	June 1	June 10	May 1	May 10	May 20	June 1	June 10
Redwing.....	36.4	36.7	36.0	35.7	34.6	34.7	35.1	35.4	34.7	35.1
Bison.....	37.8	37.3	37.1	36.4	35.5	36.1	36.7	36.4	36.1	36.7
Buda.....	36.7	36.8	35.3	35.3	35.0					
Winona.....	34.4	34.5	34.3	34.2	33.8					
Average.....	36.3	36.3	35.7	35.4	34.7	35.4	35.9	35.9	35.4	35.9

Variety	IODINE NUMBER									
	May 1	May 10	May 20	June 1	June 10	May 1	May 10	May 20	June 1	June 10
Redwing.....						175.2	176.4	175.3	174.8	181.0
Bison.....						164.6	159.4	158.2	156.4	172.2

Variety	DAYS FROM FULL BLOOM TO RIPE									
	May 1	May 10	May 20	June 1	June 10	May 1	May 10	May 20	June 1	June 10
Redwing.....	46	42	37	32	35	32	26	30	34	-----
Bison.....	42	42	36	35	32	32	30	30	28	-----
Buda.....	42	42	38	35	34	29	31	29	30	-----
Winona.....	42	43	38	32	32	33	29	29	34	-----
Average.....	43	42	37	34	33	32	29	29	32	-----

SUMMARY

The experimental results reported here include two phases of a study with flax—(1) the rate of development of the seed, boll, and oil on soils of different productivity, and (2) the influence of delayed planting on yield and composition of the seed.

In the first phase of the study two varieties of flax, Bison (Minnesota accession No. 199) and Diadem (Minnesota accession No. 197), were grown at University Farm, St. Paul, Minn., on a portion of the wheat plots of a 5 and 3 year rotation and on a plot continuously cropped to wheat.

Daily measurements were made of the length, width, and thickness of the seed, length and diameter of the boll, and dry weight of the seed of both varieties. The rate of development of the oil content was studied only with Bison. The iodine number was determined on the seed obtained from Bison grown on the 3-year-rotation plot.

The daily seed and boll measurements indicate a more rapid rate of growth during the first week after flowering on the soil of lowest productivity (the continuous cropping plot). After the maximum seed and boll size was reached there was no consistent difference in size of seed or boll on either of the plots used.

The measurement of the daily increase in dry weight of the seed gave the same type of response on soils of different productivity during the period of most rapid increase in seed weight. From this period to maturity (approximately seven days) the seed of Diadem grown on the continuous-cropping plot was found to be significantly smaller than from the 3 or 5 year rotation plots. The seed weight

of Bison during a similar period was practically the same from each of the plots, which suggests a varietal response to soil fertility, Bison probably being better suited than Diadem to soil of low productiveness.

Varietal difference in rate of development of seed and boll and the dry weight of the seed were studied from the averages from the three plots. The growth curves show that the length, width, and thickness of the seed and the length and diameter of the boll increased rapidly, reaching an approximate maximum size for both varieties at nine days after flowering, remained fairly constant for a considerable period, then decreased slightly with complete maturity.

The daily increase in dry weight of the seed progressed at a fairly uniform rate until 24 days after flowering for Bison and 28 days after flowering for Diadem, then increased slightly to complete maturity. The results of the measurements show that the growth rate of both varieties are nearly identical.

The average oil content of Bison, studied at 2-day intervals, showed a fairly uniform increase from 5 days to 25 days after flowering, followed by a slight decrease to maturity.

The iodine absorption number made at frequent periods on the seed of Bison from the 3-year rotation suggests that the saturated fatty acids are formed first in the synthesis of oil, but are progressively transformed to acids with a greater degree of unsaturation. The iodine numbers increased rapidly from 5 days to 17 days after flowering, remained fairly constant for a short period, then decreased slightly to complete maturity.

In the second part of the study, which dealt with the influence of delayed planting on the yield and composition of the seed, four standard varieties were grown during 1929 and 1930 in replicated rod-row plots and were sown at five successive 10-day intervals beginning on May 1. From these varieties the yield of seed, average number of seeds per boll, average number of bolls per plant, percentage of oil, and the iodine absorption number of the oil were determined from each planting date.

The results of this phase of the study show that the yield of seed is greatly reduced as a result of delayed planting. The reduction in the number of seeds per boll and the number of bolls per plant followed very closely the reduction in yield, which may explain the lowered yield from late-planted flax. The reduction in the number of seeds per boll could not be attributed to lack of fertilization of the flower, since rudimentary seeds were present in the ovary. High temperatures may have been lethal to the developing seed at an early stage of growth when its moisture content was very high.

The analysis of the seed showed a very slight reduction in oil content with delayed planting in 1929, but no consistent differences in 1930. The iodine number of the oil determined from two varieties in 1930 indicated a slight decrease with delayed planting for Bison but no significant change for Redwing.

The results of this study suggest a difference in varietal response to delayed planting. Of the four varieties tested, Redwing and Winona showed less reduction in yield than Bison and Buda.

LITERATURE CITED

- (1) ANONYMOUS.
1908. THE PROBABLE ERROR OF A MEAN. By Student. *Biometrika* 6: 1-25, illus.
- (2) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1925. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. REVISED TO JULY 1, 1924. Ed. 2, 535 p., illus. Washington, D. C.
- (3) BUSHEY, A. L., PUHR, L., and HUME, A. N.
1927. A STUDY OF CERTAIN PHYSICAL AND CHEMICAL CHARACTERISTICS OF FLAXSEED AND OF LINSEED OIL. *S. Dak. Agr. Expt. Sta. Bul.* 228, 11 p.
- (4) COLEMAN, D. A., and FELLOWS, H. C.
1927. OIL CONTENT OF FLAXSEED, WITH COMPARISONS OF TESTS FOR DETERMINING OIL CONTENT. *U. S. Dept. Agr. Bul.* 1471, 35 p., illus.
- (5) DILLMAN, A. C.
1928. DAILY GROWTH AND OIL CONTENT OF FLAXSEEDS. *Jour. Agr. Research* 37: 357-377, illus.
- (6) EYRE, J. V., and FISHER, E. A.
1915. SOME CONSIDERATIONS AFFECTING THE GROWING OF LINSEED AS A FARM CROP IN ENGLAND. *Jour. Agr. Sci. [England]* 7: [120]-134, illus.
- (7) FABIAN, H.
1928. DER EINFLUSS DER ERNÄHRUNG AUF DIE WERTBESTIMMENDEN EIGENSCHAFTEN VON RASTFASERPFLANZEN (FLACHS UND NESSEL) UNTER BESONDERER BERÜCKSICHTIGUNG DER AUSBILDUNG IHRER FASERN. *Faserforschung* 7: 1-56, [69]-115, illus.
- (8) GARNER, W. W., ALLARD, H. A., and FOUBERT, C. L.
1914. OIL CONTENT OF SEEDS AS AFFECTED BY THE NUTRITION OF THE PLANT. *Jour. Agr. Research* 3: 227-249.
- ✓(9) HAYES, H. K., and GARBER, R. J.
1927. BREEDING CROP PLANTS. Ed. 2, 438 p., illus. New York.
- (10) IVANOW, S.
1912. ÜBER DEN STOFFWECHSEL BEIM REIFEN ÖLHALTIGER SAMEN MIT BESONDERER BERÜCKSICHTIGUNG DER ÖLBILDUNGSPROZESSE. *Bot. Centbl., Beihefte* (1) 28: [159]-191, illus.
- (11) KAYSER, R.
1925. HAT EINE MINERALDÜNGUNG EINFLUSS AUF DIE WERTBESTIMMENDEN EIGENSCHAFTEN VON ÖLPFLANZEN UND ÄNDERT SICH DURCH DIE DÜNGUNG DAS ÖL IN SEINER ZUSAMMENSETZUNG? *Bot. Arch.* 10: 349-386, illus.
- (12) RABAK, F.
1918. INFLUENCE ON LINSEED OIL OF THE GEOGRAPHICAL SOURCE AND VARIETY OF FLAX. *U. S. Dept. Agr. Bul.* 655, 16 p.
- (13) STARK, R. W.
1924. ENVIRONMENTAL FACTORS AFFECTING THE PROTEIN AND THE OIL CONTENT OF SOYBEANS AND THE IODINE CONTENT OF SOYBEAN OIL. *Jour. Amer. Soc. Agron.* 16: 636-645.
- (14) TOBLER, F.
1928. DER FLACHS ALS FASER- UND ÖLPFLANZE. 273 p., illus. Berlin

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 45

WASHINGTON, D. C., SEPTEMBER 1, 1932

No. 5

RELATION OF COMMERCIAL HONEY TO THE SPREAD OF AMERICAN FOULBROOD¹

By A. P. STURTEVANT²

Associate Apiculturist, Division of Bee Culture, Bureau of Entomology, United States Department of Agriculture

INTRODUCTION

The relation and importance of commercial honey to the spread of American foulbrood of bees has occupied the attention of the bee-keeping industry more or less prominently for many years. The theory has been promulgated that honey which has not come from disease-free apiaries is dangerous because of the possibility of its disseminating American foulbrood. A few States and at least one foreign country require that honey intended for interstate shipment be accompanied by a certificate from the bee inspector of the State in which the honey originated to the effect that such honey was produced in apiaries free from American foulbrood.

It is a well-established fact that honey taken directly from the combs of the brood chambers of colonies affected by American foulbrood is capable of producing the disease if fed to healthy colonies. Since commercial beekeeping practice bans the extracting of honey from the brood nest, it is difficult to understand how heavily infected honey, in large quantities, could get on the market. Whether honey from supers that have been on colonies affected with American foulbrood is of serious importance in transmitting the disease is still open to question. White (30, p. 35)³ says: "The likelihood that the disease will be transmitted by combs from diseased colonies, which contain honey but no brood, probably is frequently overestimated." On the other hand, Millen (23) found that combs built from foundation and completely filled above an excluder with honey from colonies that had been destroyed by American foulbrood produced disease in all of 10 colonies made from package bees to which one comb each of the honey had been given. Corkins (8) expressed the belief, as a result of preliminary studies, that "Extracted honey produced above an excluder in a colony in the early stages of American foulbrood is insignificant in the spread of this disease through commercial honey." The conflicting nature of these observations emphasizes the need for further research before the certification of honey is required as a means of alleviating the foulbrood situation.

In both animal and plant disease bacteriology it is known that pathogenic microorganisms may vary considerably, even within indi-

¹ Received for publication Feb. 1, 1932; issued September, 1932.

² For advice and assistance the writer is indebted to Prof. C. L. Corkins and C. H. Gilbert, of the University of Wyoming; Prof. R. C. Richmond, Deputy State entomologist, apiary investigations, Colorado Agricultural College; H. Rauchfuss, of Englewood, Colo.; N. L. Henthorne, of Greeley, Colo.; and C. H. Ranney, of Lander, Wyo. Appreciation is also expressed for the many courtesies extended by H. C. Hilton, supervisor of the Medicine Bow National Forest.

³ Reference is made by number (italics) to Literature Cited, p. 284.

vidual species, in virulence and in ability to produce disease. Furthermore, as stated by Zinsser (31, p. 188-189)—

Whether or not infection occurs depends also upon the *number of bacteria* which gain entrance to the animal tissues. A small number of bacteria, even though of proper species and of sufficient virulence, may easily be overcome by the first onslaught of the defensive forces of the body. Bacteria, therefore, must be in sufficient number to overcome local defenses and to gain a definite foothold and carry on their life processes, before they can give rise to an infection. The more virulent the germ, other conditions being equal, the smaller the number necessary for the production of disease. The introduction of a single individual of the anthrax species, it is claimed, is often sufficient to cause fatal infection; while forms less well adapted to the parasitic mode of life will gain a foothold in the animal body only after the introduction of large numbers.

In the case of American foulbrood the quantity of infectious material that honey must carry in order to produce disease in a colony has never been determined. White (30, p. 20, footnote 1) states, in connection with inoculating healthy colonies experimentally with *Bacillus larvae*:

It was found that less than one scale is sufficient disease material to produce a considerable amount of disease in the colony. In some experiments one scale, therefore, might supply all the spores needed although the use of a somewhat greater quantity of material is advisable in most instances.

While infected honey no doubt does become mixed with disease-free honey, it is probable in many cases that, because of the practice of using large settling and storage tanks, infected honey would be so diluted with spore-free honey as to make the spore content insufficient to produce infection even if fed to healthy bee larvae. Therefore, one object of these investigations was to determine the minimum number of spores of *Bacillus larvae* in honey necessary to produce American foulbrood in healthy colonies of bees as correlated with the infectivity or spore content of the average commercial honey.

In order to obtain information relative to this subject, experiments were conducted in the apiary over a period of five years. In these experiments honey or sugar sirup with a known content of spores of *Bacillus larvae* was fed to healthy colonies and the minimum number of spores that would produce infection was determined. At the same time laboratory studies were carried on with cultures of spores of *B. larvae*, concerning certain growth phases of the organism, particularly the minimum number of spores that would produce vegetative growth on artificial culture media. Methods for demonstrating the presence or absence of spores of *B. larvae* in samples of commercial honeys were also investigated, and these honeys were studied in relation to their infectiousness as correlated with the spore-feeding experiments. These three phases of the investigation will be discussed in the order mentioned.

MINIMUM NUMBER OF SPORES OF BACILLUS LARVAE NECESSARY TO PRODUCE DISEASE IN HEALTHY COLONIES OF BEES

METHODS OF PROCEDURE

LOCATION OF EXPERIMENTS

These investigations were started during the summer of 1926 in a small experimental apiary located about half a mile from the bee culture laboratory of the Bureau of Entomology at Somerset, Md. The location at Somerset was undesirable, however, because of its

close proximity to the apiary connected with the laboratory and to other privately owned colonies of bees, necessitating extreme precautions to prevent spread of the disease. In 1927 the experimental work was transferred to the Intermountain States bee culture field laboratory at Laramie, Wyo.⁴ In Wyoming an ideal isolated location was found about 14 miles east of Laramie in the Medicine Bow National Forest, the nearest colonies of bees being at least 14 miles away and probably farther. Since this location is more than 8,000 feet above sea level, there is only a slight nectar flow from wild flowers, which assures the immediate use of any inoculated sirup fed to colonies of bees. In fact, after the middle of the summer it was found necessary in most cases to feed the experimental colonies with uninoculated sugar sirup in order to prevent starvation.

In 1927 and 1928 the colonies used for experimentation were located in two yards between a quarter and a half mile apart. The arrangement of the colonies in the two yards was such as to prevent drifting as much as possible. In 1929 and 1930, in order to limit still further the danger of transmission of disease because of drifting or robbing, 20 colonies were stationed in pairs, so arranged as to minimize the danger from drifting, in 10 isolated locations at least a quarter of a mile apart.

MAKE-UP OF COLONIES

Five-frame nucleus hives were used for the spore-feeding experiments. The colonies were prepared either with two or three frames of brood, honey, and adhering bees taken from healthy colonies, together with a young laying queen, or, as in 1927, 1928, and 1929, by placing a 2-pound package of bees containing a laying queen on foundation or on combs containing honey from healthy colonies and feeding them sugar sirup. During a good honey flow these small colonies were allowed to build up in the apiary connected with the laboratory until they consisted of three or four frames of brood before they were moved to the isolated locations. The bees making up the colonies used for the feeding experiments from 1927 to 1930 at Laramie, Wyo., were all from the same general strain.

MATERIAL USED FOR INOCULATION

Spores of *Bacillus larvae* were obtained from American foulbrood scales in combs taken from diseased colonies located in the States of Maryland, Iowa, and Wyoming. The strain used at Somerset, Md., was obtained from a sample sent to that laboratory for diagnosis. Two different strains were used at Laramie during 1927, 1928, and 1929, one obtained from a diseased colony in the experimental apiary belonging to the University of Wyoming and one obtained from a beekeeper at Lander, Wyo. In 1930 three other strains were used in the feeding experiments, one from Iowa and two from apiaries in Wyoming.

PREPARATION OF SPORE SUSPENSIONS

In preparing the spores for feeding to the healthy colonies, scales were removed from the combs by means of sterile forceps (the necessary precautions being taken against contamination) and placed in

⁴ This laboratory is maintained cooperatively by the University of Wyoming and the U. S. Department of Agriculture.

a flask containing 50 c c of sterile water and glass beads. After the scales had softened in the water, the flask was shaken for one-half hour to insure complete maceration of the scales. The suspension was then filtered through two thin layers of sterile absorbent cotton into another sterile flask in order to remove any lumps or débris.

In preparing the stock suspensions of spores, at first 75 to 100 scales were taken by counting. Later it was found that the average American fowlbrood scale weighs 0.0223 g. Therefore, the 100 scales for the stock suspensions were obtained by weight, the scales being weighed in a sterile covered glass dish before they were deposited in the flask of sterile water.

After the suspension had been filtered and tested for contamination and was ready for use, the number of spores per cubic centimeter was determined by the following method: By means of a blood-diluting pipette giving a dilution of 1 to 20, the spore suspension was diluted with a weak solution of carbol fuchsin and a drop placed in the counting chamber of a Helber bacteria-counting cell 0.02 mm deep and ruled in squares of 0.0025 mm² each.⁵ With the use of two 15× eyepieces in a binocular microscope and a 1.8-mm oil-immersion objective, the spores in 25 squares of the Helber chamber were counted. Then by means of the formula

$$\frac{\text{Total spores counted} \times \text{dilution} \times 20,000 \times 1,000}{\text{Number of squares counted}}$$

the approximate number of spores per cubic centimeter in the suspension was determined.

Later this method was checked by the method of Breed and Brew (2) for counting bacteria in milk. With the aid of a binocular microscope having two 15× eyepieces and a 1.8 mm oil-immersion objective, the area of a circle etched on an ocular micrometer disk was determined by means of a stage micrometer. One one-hundredth cubic centimeter of a 1 to 100 dilution of the stock suspension of spores was placed on a glass slide on which 1 cm² had been ruled with a diamond pencil. This was mixed with a small loopful of carbol fuchsin stain and the whole spread over the 1 cm² of surface⁶ and allowed to dry uniformly. The number of spores per cubic centimeter of the stock suspension was determined according to the formula

$$\frac{\text{Area 1 cm}^2}{\text{Area of circular field}} \times \frac{\text{total number of spores counted} \times \text{dilution} \times 100.}{\text{number of circular fields counted}}$$

These two methods were found to check fairly closely within the limits of the precision of the methods used in counting. Furthermore, by both methods it was found that in the majority of cases 100 scales in 50 c c of water give approximately 5,000,000,000 spores per cubic centimeter for each suspension made up in this way. Therefore, this number was used as a standard for making all dilutions.

⁵ Mm² and cm² are the abbreviations for square millimeter and square centimeter, respectively, recently adopted by the Style Manual for United States Government printing.

After a considerable number of counts had been taken in making up several stock suspensions of spores, counting was eliminated and the spore content of the stock suspensions was standardized according to the method described by Gates (11, p. 114), as follows: "The opacity of a bacterial suspension is measured by the length of a column of the suspension required to cause the disappearance of a wire loop." An instrument known as a suspensiometer was used for this purpose. The use of this method saved considerable time and labor without appreciably affecting the precision of the counts. One liter of a 50 per cent solution of sugar in water was used as the standard quantity of inoculated sirup fed to each experimental colony. A series of dilutions of the original stock suspension containing 5,000,000,000 spores was made by adding different quantities of the spore suspension to 1 liter of sugar sirup. In this way the approximate total number of spores in each liter of sugar sirup to be fed to colonies of bees was known.

METHOD OF INOCULATING COLONIES

In 1926 at Somerset, Md., the sugar sirup containing the various dilutions of spores was fed to the colonies by means of galvanized-iron troughs that were hung inside the hives after two combs had been removed. In these troughs sterile excelsior was placed for the bees to walk on in order to prevent them from drowning. This method was found unsatisfactory, however. At Laramie, Wyo., the sugar sirup containing the spores was first placed in Boardman feeders, but owing to the danger of robbing at the entrance of the hives, the method finally used was to invert the jars in holes bored in the hive covers. In this way any leakage into the hives was cleaned up by the bees without danger of causing robbing. To prevent the jars from being broken or knocked over, box covers were placed over them and fastened to the hive covers. Each colony was usually inoculated only once with an individual dilution of spores. Duplicate colonies were inoculated with each dilution of spores. Uninoculated check colonies were placed among those that were inoculated.

PRIMARY OBSERVATIONS

Observations of the condition of the brood were made at least once a week, and sometimes oftener, after the colony was given the liter of inoculated sirup. In 1926 at Somerset, Md., as soon as diseased larvae appeared in a colony, the colony was killed and at once removed from the apiary. Because of the isolated location near Laramie, Wyo., the colonies were left until the end of the brood-rearing season, when final observations were made.

The results of the spore-feeding experiments are shown in Table 1.

TABLE 1.—Results of spore-feeding experiments *

[Duplicate colonies of bees (A and B) were used in the first 4 years, and triplicate colonies (A, B, and C) in 1930]

Total number of spores fed	Extent of foulbrood in—																		
	1926		1927				1928, repeat		1928				1929				1930, final		
			During season		Final				During season		Final		During season		Final				
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	C		
5,000,000,000	+	?																	
2,500,000,000	+	+							+	+	+	+							
1,000,000,000	+	+							+	+	+	+							
750,000,000	+	+																	
500,000,000	+	+	+		+	+			+	+	+	+	+	+					
350,000,000	+	0			+	+			+	+	+	+							
200,000,000	+	?	+	+	+	+	+												
175,000,000			+	+	+	+	+												
150,000,000			?	+	+	+	+												
125,000,000			0	?	0	+	+	0	+	+	+	+							
100,000,000	0	0	0	?	+	+	+	0	+	+	+	+	+	0	+	+	0	0	
75,000,000			+	?	+	+	+	+	+	+	0	+	+	+	+	+	0	0	
50,000,000	0	0	0	0	0	0	0	0	0	+	0	+	+	0	+	+	0	0	
25,000,000													0	0	0	0	0	0	
10,000,000	0	0											0	0	0	0	0	0	
5,000,000													0	0	0	0	0	0	
2,500,000													0	0	0	0	0	0	
1,500,000													0	0	0	0	0	0	
500,000													0	0	0	0	0	0	
100,000													0	0	0	0	0	0	
Controls	1+,12-0		1+,2-0		1+,2-0		1-0		1+,3-0		1+,3-0		8-0		8-0		2-0		

* +, Positive American foulbrood; ?, probable American foulbrood, very slight and unconfirmed and disappearing by end of brood-rearing season; 0, no disease found during season; -, disease cleaned out by end of brood rearing; —, no recurrence in second season.

In 1926 a total of 200,000,000 spores fed to a colony was the smallest number that produced disease; in 1927, on the other hand, 75,000,000 was the smallest number. However, in the latter year the spores were obtained from another locality in which environmental conditions were quite different. In an effort to obtain check results, the feeding experiments were repeated in 1928. Through an error in making up the spore dilutions, which was not discovered until too late for rectification, no colony received less than 50,000,000 spores. This season one colony of the pair receiving an inoculation of 50,000,000 spores became infected. The feeding experiments were repeated again in 1929, with dilutions of spores from 75,000,000 down to 100,000—considerably less than the minimum number in 1928. Again only one colony of the pair receiving a total of 50,000,000 became infected. As a result of two years' experiments this was found to be the apparent minimum number of spores of *Bacillus larvae* capable of producing infection when fed in 1 liter of sugar sirup. In 1930 spores from three different localities were fed in duplicate to six healthy colonies in dilutions of 50,000,000 and 25,000,000 without producing disease.

It is therefore apparent that a certain minimum number or mass of spores is required to start the initial action capable of producing American foulbrood in healthy larvae. Under the conditions of these experiments this minimum number was approximately 50,000,000 spores of inoculum per liter of sirup.

SECONDARY OBSERVATIONS

During the first three years of the experiments, or previous to 1929, at which time the experimental colonies were isolated in pairs, certain of the uninoculated control colonies developed disease, 1 out of 13 in

1926, 1 out of 3 in 1927, and 1 out of 4 in 1928. It was assumed that the disease was probably not spread by robbing, since no active robbing was observed at any time. In practically every case where a control colony became infected, it was so located in relation to the inoculated colonies that drifting of young nurse bees during play flights could account for the spread of the disease, in one or two cases quite definitely so. In 1929 all eight uninoculated colonies, although they were not located with the inoculated colonies but were within robbing range of all, remained free from disease. The prevention of drifting apparently eliminated the casual spread of disease.

Occasionally a colony of bees affected with American foulbrood will try to clean out the diseased remains, often removing parts of the scales and sometimes actually tearing a comb down to the midrib in order to do this. White (30, p. 34-35) states:

There is considerable evidence to support the belief that occasionally in cases of light infection the disease may disappear unaided by treatment. * * * It should be emphasized that such a course for the disease, if it occurs at all, is unusual. Although American foulbrood spreads more or less rapidly within an infected colony, the fact remains that it frequently does not.

Lineburg (16) in 1925 reported that in two colonies which were diseased in the spring the disease apparently disappeared later in the season. Three colonies were divided and used for making increase in June and July, but all remained free from disease, at least until the end of that season. Further observations were not reported. Corkins (8) in 1928 reported five colonies which were given combs containing scales of American foulbrood at the beginning of the honey flow of 1927 and developed no disease up to July 10, 1928. Two other colonies were observed to have cleaned out the disease and remained healthy for an entire season. However, during the several years of his experimental work on American foulbrood, the writer never observed a colony in which the disease was permanently cleaned out until 1927. In that year, of 16 colonies inoculated with various dilutions of spores, 4 colonies, 2 of which received more than the probable minimum dose causing infection, showed no disease during the season. The disease completely disappeared by the end of brood rearing in 10 of the 12 other colonies that had showed either positive or probable disease some time during the summer. In 1928 package bees were placed on the combs of seven of these colonies that had apparently cleaned out the disease during the previous summer and on two that had been inoculated with presumably a sufficient number of spores but which had remained healthy. Three of the seven developed disease again the second season, while four remained healthy during the entire season. Neither of the two inoculated colonies that had remained free from disease in 1927 developed it in 1928. Of the 11 colonies inoculated in 1928 that developed disease, 4 cleaned up the disease by the end of the brood-rearing season and 2 inoculated colonies showed no disease. In 1929, 1 of the 2 colonies developing disease cleaned up by the end of the brood-rearing season, making a total of 15 cases in which the disease was cleaned up by the end of brood rearing. Two of the colonies inoculated with the minimum infectious dose or more showed no disease during that summer.

It is possible that, in the high altitude of Laramie, and in similar places where the air is very dry, the scales of American foulbrood

become dried without adhering so tenaciously to the cell walls as they do in more humid climates at lower altitudes. These observations indicate the necessity of further work on the resistance of bees to the disease and variation in virulence of different strains of the organism.

INOCULATION OF INDIVIDUAL BEE LARVAE WITH DEFINITE NUMBERS OF SPORES OF *BACILLUS* LARVAE

In the light of the results of the foregoing experiments, in which colonies were inoculated with presumably a quantity of spores sufficient to produce infection but in which no disease developed, the question arises as to what became of the spores in the sugar sirup, some of which presumably were fed to healthy larvae. In those colonies developing disease that received a minimum number of spores, how many spores did each larva developing the disease receive? In order to obtain information on these points, a preliminary series of experiments was planned in which individual larvae were inoculated with known numbers of spores.

Toumanoff (29) reports that he was unable to cause infection by giving individual larvae a drop of a rich emulsion of a culture of *Bacillus larvae* in salt solution. He found that many of the larvae so treated were removed from the cells by the bees, and those remaining failed to develop disease. He further found that larvae given only uninoculated salt solution were also removed in the same way. Therefore, in the present experiments sugar sirup was used instead of salt solution. In a comb from a healthy colony containing numerous coiled larvae, a drop of an uninoculated 50 per cent solution of sugar in water was placed in each cell containing a larva, as near the mouth parts of the larva as possible. The rim of each cell so treated was marked with a paint consisting of 1 part of liquid white shellac, 1 part of a paint pigment, and 4 parts of ethyl alcohol. The sugar sirup was slightly colored with water-soluble eosin in order to aid in determining the effect. Frequent observations showed that practically all larvae that were fed this colored sugar sirup developed normally and were sealed over, the pigment markings still being present on the edges of the cappings. In most of the cells a residue of colored sirup could be observed for several hours after the larvae had fed.

A series of 5-frame nuclei was prepared, each containing one or two combs having a large number of unsealed larvae. A set of dilutions of spores was made from a stock suspension with a sterilized 50 per cent sugar sirup in such a way that each 0.01 c c of the dilution would contain an approximate known number of spores, as indicated in Table 2. Sterilized 2 c c Luer tuberculin hypodermic syringes graduated in 0.01 c c, the needles of which had been blunted, were used in inoculating the cells containing coiled larvae. Fifty or more coiled larvae at least 4 days old were each given 0.01 c c of a dilution of spores, each dilution being given to larvae in one comb in a separate colony, and the cells so inoculated were distinctively marked. A few larvae that had just been sealed also were inoculated by puncturing the capping with the inoculating needle and depositing the 0.01 c c in the cell. Observations were taken at the end of 24 hours and at frequent intervals thereafter until the end of the brood-rearing season.

TABLE 2.—Results of inoculation of individual healthy bee larvae with known numbers of spores of bacillus larvae, 1930

Spores fed each larva in 0.01 cubic centimeter of dilution	First inoculation, July 14		Second inoculation, Aug. 1			Third inoculation, Aug. 19				Fourth inoculation, Aug. 21							
	Colony No.	Larvae developing disease Aug. 1	Colony No.	Larvae developing disease		Colony No.	Length of time isolated from nurse bees	Effect on larvae of being isolated from nurse bees	Larvae developing disease			Colony No.	Length of time isolated from nurse bees	Effect on larvae of being isolated from nurse bees	Larvae developing disease		
				Aug. 19	Oct. 7				Aug. 26	Sept. 9	Sept. 19				Oct. 7	Aug. 26	Sept. 9
Number	Number	Number	Number	Number	Number	Hours			Number	Number	Number	Minutes			Number	Number	Number
50,000,000	12	0	14	0	0	1	Larvae removed.		0	0	0	5	Larvae removed.		0	0	0
20,000,000	13	0	34	0	0	1	do.		0	0	0	10	do.		0	0	0
10,000,000	20	0	18	0	0	3/4	Larvae not removed.		0	0	0	13	Larvae not removed.		0	0	0
7,500,000	15	0	22	0	0	1	Three-fourths of larvae removed.		0	13	0	15	do.		0	9	0
5,000,000	4	0	28	0	0	3/4	One-half of larvae removed.		0	0	0	20	do.		0	0	0
1,000,000	15	0	16	0	0	1	Larvae not removed.		0	0	0	30	One-half of larvae removed.		0	0	0
500,000	13	0	16	0	0	1	do.		0	0	0						
250,000	18	0	22	0	0				0	0	0						
100,000	22	0	0	0	0												
75,000	26	0	0	0	0												
50,000	28	0	0	0	0												
25,000	4	0	0	0	0												
10,000	15	0	0	0	0												
7,500	20	0	0	0	0												
5,000	12	0	0	0	0												
1,000	13	0	13	0	0												
750	14	0	0	0	0												
500	15	0	0	0	0												
250	18	0	0	0	0												
100	22	0	0	0	0												
75	26	0	0	0	0												
50	28	0	0	0	0												
25	4	0	0	0	0												
10	15	0	0	0	0												
5	20	0	0	0	0												
1	34	0	0	0	0												
0	32	0	0	0	0												

* Number of cells showing American foul-brood remains.

In the first series of inoculations the number of spores fed each larva ranged from approximately 5,000 down to 1. None of the larvae inoculated developed disease. (Table 2.) Later a second series of inoculations was made. The same colonies were used because of the limited number available, but the larvae inoculated were in a different comb in each colony and a different color was used to mark the cells. In these inoculations the number of spores fed ranged from 5,000,000 down to 1,000 per larva. No disease developed from this set of inoculations.

It was thought possible that the nurse bees might be removing most, if not all, of the inoculated sugar sirup before the larvae had had time to ingest a sufficient number of spores to bring about infection. Therefore, in a third series of experiments each inoculated comb was placed in a screen-wire queen-nucleus introducing cage, and this cage was put back in the colony for periods ranging from one-half to one hour before the unprotected comb was replaced in the colony, thus theoretically giving the larvae time to ingest some of the sugar sirup before the nurse bees had access to the inoculated cells. In these tests the larvae were kept from the bees so long that many of them, becoming hungry, were starting to crawl from the cells. The number of spores fed ranged from 50,000,000 down to 500,000 per larva. Twenty-four hours after the larvae were fed it was found that all receiving 50,000,000 and 25,000,000 spores had been removed from the cells, while those receiving a smaller number of spores were either partly removed or remained in the cells, according to the strength of the dilution and the length of time that the larvae were kept away from the nurse bees. (Table 2.)

Two days later another set of larvae was inoculated with the same dilutions as were previously used for these colonies but on the other side of the same combs. In this series the combs were kept away from the bees for periods ranging from 5 minutes for the heaviest dilution to 30 minutes for the weakest. Again all the larvae receiving the 50,000,000 and 25,000,000 spores were removed, while those receiving the 5,000,000, which were kept from the bees for half an hour, were partly removed, and those receiving 7,500,000 or 10,000,000 were not removed. Apparently there are two factors concerned in the removal of the larvae—the length of time they are kept away from the bees and the amount of foreign matter in the sirup, as indicated by the spore content, that is given to the larvae.

The results of the last two series of inoculations showed that in the colonies in which the larvae were not removed, or were not entirely removed, several larvae in the colony receiving 10,000,000 spores per larva developed disease, while those in the colonies receiving a smaller number remained healthy. (Table 2.) This work should be repeated with a different colony for each set of inoculations, although apparently the disease did not spread in the colonies used. Only one colony of the entire number developed disease. Although a certain degree of success was obtained, these results seem to bear out Toumanoff's (29) conclusion that the artificial infection of individual larvae is not brought about so easily as one had been in the habit of believing. Apparently, also, a considerable number of spores are necessary to establish an infection under these conditions.

MINIMUM NUMBER OF SPORES OF *BACILLUS LARVAE* PRODUCING VEGETATIVE GROWTH ON ARTIFICIAL CULTURE MEDIA

Bacteria are known to pass through a definite cycle of growth, particularly when cells from an old culture are transferred to fresh culture media. The growth stages have been described by Buchanan (3; 13, Ch. V), Henrici (12), and Winslow (13, Ch. VI) somewhat as follows: The initial stationary phase during which no growth takes place; the logarithmic phase when the organisms begin to divide, slowly at first but gradually accelerating; and so on through the complete cycle of growth. Henrici (12, p. 21, 24) has observed that—

Various factors, as temperature; the size, the age, and previous history of the inoculum; and the composition and nutrient value of the medium, influence the growth curves of bacteria. * * * Of the various factors which influence the rate of growth and form of the growth curve, the initial number of cells introduced into a unit volume of medium seems to be one of the most important.

Robertson (25), in studies of cultures of certain protozoa, has shown that growth seems to be stimulated by the presence of other cells of the same type. This characteristic has been described at various times as mass action or communal activity.

Early in 1929, in conjunction with the spore-feeding experiments in the apiary, an investigation was started to determine whether there is a similar manifestation of mass action in the vegetative growth of spores of *Bacillus larvae* on artificial culture media. In a preliminary paper on this subject the writer (27, p. 456) made the following observations: Starting with a seeding of 5,000,000,000 spores of *B. larvae* on a suitable slanted solid culture medium, it was found at the end of 48 hours' incubation at 37° C. that growth had occurred in the original and in a diluted seeding containing 60,000,000 spores, but not in one containing 50,000,000 spores. Growth occurred in a diluted seeding containing only 5,000,000 spores after six days' incubation, and in one containing 700,000 spores after 10 days' incubation. (Table 4, Group 1.) These observations indicated that a certain initial mass of spores is necessary to start vegetative growth. Furthermore, although the growth results were rather irregular owing to the comparatively small number of cultures made, they seemed to show that, within certain limits, the smaller the seeding the longer the incubation period necessary to obtain germination of the spores and vegetative growth. From this preliminary work it was assumed that the lower limits of dilution of the stock suspension that would give growth on longer incubation had not been reached.

Ahrens (1) has observed, in cultural studies of scales treated with formalin solution for different lengths of time, that growth may occur in cultures from such scales after varying periods of incubation up to 30 days, depending on the length of treatment and the percentage of formalin in the solution. Burnside (7) states, in connection with studies of disinfection of American foulbrood combs by fumigation with formaldehyde gas, that "it is probable that if scales had been washed and the incubation period increased, growth of *Bacillus larvae* would have been obtained in some instances when negative results were recorded."

Therefore, a single trial series of cultures was run (No. 7, Table 4), the total incubation period being 30 days. Results from this set of cultures showed that in some cases growth was obtained after 30

days' incubation where no growth was observed after 10 days' incubation. Work on this phase of the problem was continued during the summer and fall of 1930. Several sets of cultures were made in which *Bacillus larvae* from eight different localities were used in a series of seedings with a decreasing number of spores for each lot of the organism and all incubated for 30 days. (Table 4, Group 2.)

METHODS OF PROCEDURE

CULTURE MEDIA

A culture medium was used similar to that employed by the writer in the preliminary experiments (27) and also in earlier cultural work with *Bacillus larvae* (26)—that is, a combination of the medium made of yeast-extract and egg-yolk suspension and the carrot-extract medium of Lochhead (18). The yeast-carrot extract medium was prepared as follows:

(A) Dried yeast.....	grams..	10
Peptone.....	do....	10
Buffer (sodium glycerophosphate).....	do....	2.5
Water (distilled).....	cubic centimeters..	500

This solution was heated in flowing steam for one-half hour and, after a tablespoonful of siliceous earth had been added to assist in the filtration and clarification, it was filtered through filter paper on a perforated porcelain funnel with suction.

(B) Two hundred grams of cleaned carrots was macerated in a meat grinder, added to 500 c c of distilled water, and allowed to stand for at least 30 minutes, preferably longer. The macerated carrot was removed by filtration through fine muslin, as much liquid as possible being squeezed from the mass. The filtrate was then clarified by the addition of siliceous earth and filtration in the same manner as the yeast-extract medium.

(C) The final base medium was prepared by mixing 500 c c of A with 200 c c of B and adding 700 c c of a 3 per cent solution of washed agar.

The reaction of the medium was so adjusted that when 2 c c of sterile egg-yolk suspension, prepared as described in a previous paper (26), was added to 10 c c of the yeast-carrot extract base medium by means of the apparatus shown in Figure 1, and described previously (26), the pH value was 6.8. The medium was then sterilized in the autoclave at 15 pounds' pressure (sea level) for 15 minutes. After it had cooled to 45° C., 20 drops, or about 2 c c, of the sterile egg-yolk suspension was added to each tube of medium, mixed by shaking, and the medium was then allowed to solidify in a slanting position.

The Lochhead yeast-extract medium was tried without the addition of egg-yolk suspension, but although it gave good growth with the heavier seedings of spores, the combination medium was found to give more uniform germination and heavier vegetative growth with the more dilute seedings. The addition of the carrot extract, while possibly adding somewhat to the growth-producing qualities of the medium, served in these experiments as an indicator for vegetative growth because of the ability of *Bacillus larvae* to produce nitrite in the carrot-extract medium without the addition of potassium nitrate (18).

PREPARATION OF DILUTIONS OF SPORES

The stock suspensions of spores of *Bacillus larvae* were made up as described earlier in this paper. A series of primary dilutions, each one-tenth of the preceding dilution, was then made up in sterile 125

c c flasks by adding 4 c c of a dilution to 36 c c of sterile water. The series of dilutions containing gradually decreasing numbers of spores per cubic centimeter to be used in inoculating the culture medium were then prepared as indicated in Table 4. Sterile burettes were used in adding the proper proportions of spore suspension or spore-suspension dilutions to the proper quantities of sterile water in sterile test tubes, in order to make up the desired series of dilutions containing approximately known numbers of spores.

INOCULATION OF CULTURE MEDIUM

Swann has observed that in old cultures of anthrax a considerable percentage of spores are dead and therefore never germinate. Because of the possibility that some of the spores in the stock suspensions of *Bacillus larvae* might not be viable, an effort was made to determine the approximate proportions of viable and dead spores in the stock suspensions. Since the determination of viable spores of *B. larvae* by means of plate cultures is difficult because of the opaqueness of the special culture medium that is required, an attempt was made to determine the percentage of viable spores by the differential staining method of Burke (4) as modified by Koser and Mills (15). The procedure is as follows: A small quantity of the spore suspension is spread in a thin film on a slide and allowed to dry without heating. The slide, after immersion in a solution of carbol fuchsin at room temperature for two minutes, is washed in water and decolorized with absolute acetone for a few seconds, washed again, and immersed in Loeffler's alkaline methylene blue for two minutes, washed, dried, and examined. Very few solid-staining forms were observed in any of the suspensions examined, possibly one or two spores in several fields. It was therefore assumed that the number of nonviable spores could be considered as negligible and probably within the limits of the precision of the measurements as indicated by this procedure.

One cubic centimeter of each dilution was added to duplicate tubes of the slanted solid medium by means of sterile 1 c c pipettes, each cubic centimeter of inoculum containing an approximately known number of spores of *Bacillus larvae*. After inoculation the cultures were incubated at 37° C. In order to prevent the liquid in the tubes from drying out on long incubation, from time to time, as the water of condensation evaporated, 2 or 3 c c of sterile broth similar in composition to that of the base medium, without the egg, was added to each tube by means of the apparatus shown in Figure 1. A total of 556 cultures was made during this series of experiments.

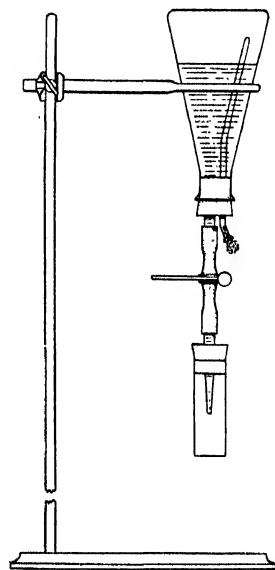


FIGURE 1.—Apparatus to replace pipetting of egg-yolk suspension

METHOD OF MAKING OBSERVATIONS

The culture tubes were incubated for 30 days at 37° C. Each tube was examined usually every 24 hours during this period. The presence or absence of vegetative growth was noted at each observation, and in cases of slight or doubtful growth the vegetative growth was checked both by microscopic examination of a stained smear and by testing for nitrite production in the culture medium by the sulphanilic acid and alpha-naphthylamine acetate test. After a large number of such observations had been made, it was found that vegetative germination of spores of *Bacillus larvae*, almost too slight to be seen, would give a definite pink color on the addition of the reagents.

Lochhead (17, p. 14) states:

It was found, however, that ordinary nitrate-reducing species, such as *B. cereus* or *Es. coli*, which are able to form nitrites readily in nitrate media, were unable to produce nitrites in recognizable amount in the peptone-carrot media, though capable of doing so upon the addition of nitrates. *Bacillus larvae* under the same condition readily forms nitrites without the addition of nitrate to the medium.

Despite this statement, a series of miscellaneous organisms was tested in standard nitrate broth, in carrot-extract broth, and on carrot-extract agar. Several organisms that commonly reduce nitrates and a few that do not were used. (Table 3.) Observations were made at short intervals during the first 24 hours. Most of these organisms gave positive nitrite tests within a few hours after inoculation in all the media used, but in the carrot-extract medium the nitrate had apparently disappeared in most cases after 24 hours' incubation, and in all cases after 48 hours. The same organisms on standard nitrate medium still gave positive tests after 48 hours' incubation. A positive nitrite test was obtained in cultures of *Bacillus larvae* that were incubated for 5 days and in one culture that was incubated for 4 days and then allowed to stand at room temperature for 16 days more before testing. Therefore, it appears probable—at least the results in Table 3 indicate—that in the case of many contaminating organisms having the power to reduce nitrite that might get into the culture tubes inoculated with spores of *B. larvae* the nitrite, if produced by the contaminating organism, would have disappeared after 48 hours' incubation, leaving contamination to be determined by gross appearance of the culture and microscopic examination. Nevertheless, in order to be sure that contaminating growth of any kind was not giving erroneous results with the nitrite test when this was used alone, any suspicious-looking growth in the culture tubes was examined under the microscope before it was tested with the reagents for nitrite production. Even though a positive nitrite test might be observed in some cases, the contaminations were recorded only as such.

OBSERVATIONS AND RESULTS

In no instance was positive growth obtained in cultures inoculated with less than 50,000 spores, even after 30 days' incubation, and growth with 50,000 spores was obtained from only two of the eight lots of spores used, namely, Nos. 19 and 23. (Table 4.) In the other six strains the minimum number of spores that produced positive growth ranged from 5,000,000 to 70,000.

TABLE 3.—Nitrate reduction by various miscellaneous organisms in standard nitrate broth and carrot-extract media during different periods of time^a

Organism	Standard nitrate broth					Carrot-extract broth							Carrot-extract agar					
	3 hours	6 hours	8 hours	24 hours	48 hours	3 hours	6 hours	8 hours	10 hours	14 hours	16 hours	24 hours	3 hours	6 hours	24 hours	48 hours	5 days	20 days
<i>Escherichia communior</i>	3+	4+	4+	4+	4+	2+	+	—	—	—	—	—	+	+	—	—	—	—
<i>Escherichia coli</i>	—	3+	4+	4+	4+	Tr.	2+	2+	—	—	—	—	+	+	—	—	—	—
<i>Escherichia typhi</i>	—	3+	4+	4+	4+	Tr.	2+	2+	—	—	—	—	+	+	—	—	—	—
<i>Alcaligenes fecalis</i>	+	3+	4+	4+	4+	—	—	—	+	—	—	—	+	+	—	—	—	—
<i>Acetobacter aerogenes</i>	3+	3+	4+	4+	4+	+	+	—	+	—	—	—	Tr.	Tr.	—	—	—	—
<i>Pseudomonas aeruginosa</i>	—	3+	4+	4+	4+	—	2+	2+	—	—	—	—	+	+	—	—	—	—
<i>Serratia marcescens</i>	—	3+	4+	4+	4+	—	+	—	—	—	—	—	+	+	—	—	—	—
<i>Shigella flexneri</i>	—	—	—	—	—	—	—	—	—	—	—	—	±	—	—	—	—	—
<i>Staphylococcus albus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Bacillus subtilis</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Bacillus pasteurianus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Bacillus mesentericus</i>	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
<i>Bacillus larvae</i>	—	—	—	2+	4+	—	—	—	—	—	—	2+	—	—	2+	4+	4+	4+

^a +, 2+, 3+, and 4+ indicate relative degrees of reaction; Tr. indicates trace; — indicates no reaction; ± indicates that the reaction was doubtful.

[illegible]

[illegible]

* Numbers in these columns refer to number of days of incubation before observations were made; + indicates vegetative growth; - indicates no vegetative growth; X indicates contamination; ± indicates that it was impossible to tell whether or not there was growth; ±± indicates that the positive was more doubtful than the negative. Where two numbers are given for a dilution, two cultures of this dilution was carried.

The length of the incubation period in relation to the decreasing number of spores used varied greatly with the different lots of spores, even with the duplicate inoculations of each lot. Table 5 gives the results of positive cultures obtained in relation to the period of incubation and the dilution of the spores. The coefficient of correlation (14, p. 179) for the positive cultures only, in relation to length of incubation and dilution of spores, was found to be 0.3558 ± 0.0440 . While this does not show a strong correlation, it indicates that with the smaller numbers of spores there is a tendency for growth to take place with longer periods of incubation. However, when the cases of positive growth were correlated with the dilution and incubation time on the basis of the percentage of positive cultures to negative cultures for each observation period of incubation time, an insignificant negative correlation was obtained. Apparently there is a variable uncontrollable factor present, more obvious when spores are used from different lots of the organism, which makes it impossible to correlate the other factors closely. However, the data summarized in Table 6 indicate that, of the 120 cultures made with seedings of between 5,000,000,000 and 9,000,000 spores per seeding, 98.33 per cent showed growth at the end of 10 days' incubation, while 100 per cent (120 cultures) showed growth after 30 days' incubation. This is 56.87 per cent of the 211 total cultures showing growth after 30 days.

TABLE 5.—Summary of the positive vegetative cultures of *Bacillus larvae* in relation to length of incubation period and with varying dilutions of the stock suspension

Number of spores per cubic centimeter inoculated	Number of positive cultures after days of incubation																				Number of cultures												
																					Posi- tive	Nega- tive Total											
	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30				
5,000,000,000	16																													16	0	16	
500,000,000	16	6	4	2																										16	0	16	
50,000,000	4	4	3	6	3																									16	0	16	
40,000,000	4	3	3	6	4																									16	0	16	
30,000,000	2	4	5	2	1	1																								16	0	16	
20,000,000		2	2	1	4	2	1																							16	0	16	
10,000,000			4		2	4		1																						14	0	14	
9,000,000			4		2	4																								10	0	10	
8,000,000			4		2	4																								8	2	10	
7,000,000			3		2	4																								5	4	9	
6,000,000			2	1	4	1	1																							8	2	10	
5,000,000			2	2	2	1	1																							8	7	15	
4,000,000							1																								8	4	12
3,000,000							1																								11	2	13
2,000,000							3	1																							7	9	16
1,000,000							1	1																							4	10	14
900,000								1																							4	10	14
800,000								2																							5	10	15
700,000																															6	7	13
600,000																															3	12	15
500,000																															2	13	15
400,000																															2	13	15
300,000																															0	15	15
200,000																															1	15	16
100,000																															1	15	16
90,000																															1	15	16
80,000																															1	15	16
70,000																															2	14	16
60,000																															3	12	15
50,000																															1	15	16
40,000																															2	14	16
30,000																															1	15	16
20,000																															2	14	16
10,000																															0	123	123
0-40,000																															0	123	123
Total positive cultures	42	21	39	13	30	9	9	1	6	3	3	2	7	0	2	2	0	0	2	2	1	0	0	1	0	1	0	0	4	211			
Total negative cultures	514	493	454	441	411	402	393	392	386	383	380	378	371	371	369	367	367	365	363	362	362	362	361	361	349	349	349	345	345	345			
Total cultures	556	514	493	454	441	411	402	393	392	386	383	380	378	371	371	369	367	365	363	362	362	362	361	361	349	349	349	345	345	345	556		

TABLE 6.—Summary of positive vegetative cultures of *Bacillus larvae* grouped in relation to size of seedings and length of incubation period

Number of spores per seeding	10 days' incubation				11 to 30 days' incubation				Final observations after 30 days' incubation						
	Num-ber of positive cul-tures	Num-ber of nega-tive cul-tures	Percent-age posi-tive	Percent-age nega-tive	Num-ber of positive cul-tures	Num-ber of nega-tive cul-tures	Percent-age posi-tive	Percent-age nega-tive	Total number of pos-itive cul-tures	Total number of nega-tive cul-tures	Total number of all cul-tures	Percent-age posi-tive	Percent-age nega-tive	Percent-age posi-tive of total of all cul-tures	Percent-age nega-tive of total of all cul-tures
5,000,000,000-	118	2	98.33	1.66					120	0	120	100	0	56.87	0
9,000,000-							1.67								
8,000,000-							18.12								
500,000	48	123	28.07	71.93	31	92	25.63	53.80	171	92	171	46.20	53.80	37.44	26.66
400,000-50,000	4	138	2.82	97.18	8	130	5.63	91.55	142	130	142	8.45	91.55	5.69	37.68
40,000-0	0	123	0	100	0	123	0	100	123	0	123	0	100	0	35.65
Total....	170	386	30.58	69.42	41	345	7.37	62.05	556	345	556			37.95	62.05

Of the 171 cultures made with seedings between 8,000,000 and 500,000 spores per seeding, 48, or 28.07 per cent, showed growth at the end of 10 days' incubation, while 79, or 46.20 per cent, showed growth after 30 days' incubation. The latter number is 37.44 per cent of the 211 total cultures showing growth after 30 days' incubation.

Of the 142 cultures made with seedings between 400,000 and 50,000 spores per seeding, only 4, or 2.82 per cent, showed growth at the end of 10 days' incubation, while 12, or 8.45 per cent, showed growth after 30 days' incubation. The latter figure is 5.69 per cent of the 211 cultures showing growth after 30 days' incubation.

Of the 123 cultures made with seedings of 40,000 or fewer spores per seeding, no growth was obtained after 30 days' incubation.

Of the 556 cultures made with all seedings, 30.58 per cent showed growth at the end of 10 days' incubation and 69.42 per cent showed no growth. The 170 positive cultures after 10 days' incubation is 80.57 per cent (not shown in Table 6) of the 211 total positive cultures obtained. In the interval between the 10 and 30 day incubation periods, 19.43 per cent (not shown in Table 6) of the 211 total positive cultures, or another 7.37 per cent of all cultures made, showed growth, making a total of only 37.95 per cent of all cultures which showed growth at the end of 30 days' incubation, with 62.05 per cent still showing no growth.

The initial growth phases as described by Buchanan (3; 13, Ch. V) are clearly more marked with spores than with simple vegetative organisms, since there is a varying length of time necessary for spores to germinate and start growing after implantation in a suitable medium. In the light of observations on other spore-forming organisms, it is probable that this factor, which seems to cause variations in the germination time of *Bacillus larvae* even within a lot from a single source, is what has been termed "dormancy." Burke (5, p. 283), working with *Clostridium botulinum*, found:

The individual (unheated) spores in a given culture of *Cl. botulinum* vary greatly in the time required for germination under optimum growth conditions. The majority germinate relatively quickly, but a few lie dormant for a longer time. One hundred and forty-four days is the maximum period of dormancy recorded here * * *.

Burke states:

The primary factors which cause the spore to lie dormant for long periods of time under optimum growth conditions are believed to be inherent in the spore itself. It is thought that relative permeability of the spore wall is one of the factors. Environmental conditions may secondarily modify the period of dormancy.

Burke, Sprague, and Barnes (6, p. 560) observed the same phenomenon with such non spore-bearing bacteria as *Bacillus coli* (= *Escherichia coli*). They found that spores of *B. subtilis* remained dormant 39 days and those of *B. megatherium* 90 days, although a large majority developed in 4 or 5 days. They believe:

Dormancy must be considered a factor in infection. It reduces the chances of infection by reducing the number of organisms that would otherwise start to grow at one time. Since the cells begin to multiply at different times, the body has an opportunity to initiate defensive reactions before all the cells develop. If dormant for a sufficient period, the organisms will be excluded from the body before development takes place.

Swann (28) has observed that there is a variation in the germination time of anthrax spores, depending on the age and condition of the spores.

Morrison and Rettger (24, p. 339) recently stated—

Because of the marked variability of germination, depending upon the stimuli supplied in the environment, the deduction is made that bacterial spores in the process of germination are vitally active bodies having requirements for metabolic function which are the same as or more exacting and specific than those of the vegetative cells.

Experimental evidence is presented to show that the dormancy of aerobic bacterial spores is largely, if not entirely, determined by conditions in the environment of the spores, and that these factors must be taken into consideration, perhaps specifically for each species, before so-called "inherent" or "normal" dormancy of bacterial spores can be established.

This phase of the work with *Bacillus larvae* is being repeated with the organism obtained from a single source in an effort to determine the importance of this variable factor of dormancy.

SPORES OF BACILLUS LARVAE IN COMMERCIAL HONEY

A few instances have been reported in the bee journals, such as that by Merrill (22), in which American foulbrood has developed as a result of bees having access to cans of infected honey that have been carelessly thrown out. Without doubt in some cases honey has been allowed to get on the market from infected colonies through negligence of the beekeepers and without being diluted by mixing or blending with honey from disease-free apiaries. On the other hand, Fracker (10, p. 379-380) has shown, by a study of disease-inspection statistics for Wisconsin:

1. In Wisconsin the introduction of this disease into the State and into many individual localities is definitely known to have been in specific importations of bees and equipment.

2. Cases of infection in which the source appears to be infected honey in the channels of trade are comparatively rare.

3. Even near such a large center as Milwaukee the infection percentage is greatest in localities of active movement, such as greenhouse areas, and is relatively low within the city itself.

4. Towns and cities of from 3,000 to 40,000 which have been natural markets for infected honey from near-by counties, have remained for years free from disease either until the present or until infected bees and equipment were introduced.

5. No new centers of infection are known to have been started since the policy of limiting movement of bees and equipment was begun in 1919.

6. These observations appear to be confirmed by conditions in the South, in spite of the fact that the period of active flight of the bee tends to continue through the peak of honey distribution.

Furthermore, F. L. Thomas, State entomologist of Texas, in an unpublished manuscript states:

The largest of the estimates with reference to the quantity of honey that is brought into Texas in a year is 19 carloads. Most of this honey is produced in California, Colorado, New Mexico, Utah, and Wyoming. * * *

If 19 carloads of foulbrood-infected honey are distributed annually in this State, it seems reasonable to suppose that our inspectors would have a hard time to keep this disease within bounds. In fact, I would expect to find that the inspectors would be gradually losing ground in their attempts to eradicate this menace. A large share of the honey which is imported is sold in west and northwest Texas where practically no bees are kept. The amount which is distributed in the beekeeping territory of the State is evidently less dangerous than is commonly supposed. The following facts, I think, will prove this statement.

During the period September 1, 1920, to August 31, 1926, the inspection work has been carried into 100 counties. Fifty-six counties were found to be free

from contagious or infectious diseases of bees, but in the other 44 counties American foulbrood has been present.

An average of 668 beekeepers have been visited each year and 38,661 colonies examined with the result that an average of 430 colonies, or 1.11 per cent, have been found to be diseased.

American foulbrood is found now in only 23 counties, 21 of the 44 counties having been cleaned up. In 12 of the counties where disease occurs, only 30 colonies were found to be infected out of 7,642 examined—less than 0.4 of 1 per cent. Six counties had one diseased colony each.

About 40 per cent of the beekeepers and 60 per cent of the colonies are re-inspected from year to year; the remainder, being free of disease and considered out of danger, are dropped and "new territory" is taken over and examined for presence of foulbrood. By "new territory" is meant beekeepers and their colonies visited and inspected for the first time. An average of 228 diseased colonies are discovered each year in "new territory." This is 1.6 per cent of the total number of colonies examined in this territory.

The reinspection which has been made in the counties where disease has been present shows that there have been both gains and losses. But a net gain has resulted which has averaged 21 beekeepers and 368 colonies freed from American foulbrood and quarantine annually.

From these facts it is easily seen that definite and really rapid progress in eradicating the disease is being made. Rarely do our inspectors find new outbreaks of disease that can not be traced to careless beekeeping methods, bees robbing infected and weakened colonies, or to the use of old and infected equipment.

It is not my intention to imply that honey is not a carrier of American foulbrood. The above evidence simply indicates that the honey which has been imported into Texas has not been as dangerous a source of disease to bees as is sometimes thought.

Practically no work has been reported on the microbiology of honey other than that in connection with the spoilage of honey through fermentation by yeasts (19, 21), and no work appears to have been done on the *Bacillus larvae* spore content of commercial honey. In 1925 the writer undertook to devise a method for demonstrating, at least qualitatively, the presence or absence of spores of *B. larvae* in honey and their significance in relation to the results of the spore-feeding experiments. Difficulties were encountered in obtaining cultures of *B. larvae* from honey. It was impossible to obtain vegetative growth of this organism, even when a considerable number of spores had previously been added to honey, because of the difficulty of eliminating contaminating organisms that developed rapidly in the honey, completely overgrowing any possible vegetative growth of *B. larvae* before it could get well started. Therefore, methods of concentrating the spores from the honey and of identifying them by means of microscopic examination were attempted. Because spores of *B. larvae* have a characteristic appearance in stained smears (20, p. 9), it was assumed that this method might give at least tentative evidence.

METHODS OF PROCEDURE

The first method attempted was the filtration of honey diluted with water through a membrane of ether-alcohol collodion or through filter paper impregnated with an acetic acid solution of collodion (9). Apparatus was devised in which both suction and pressure were tried in this filtering process. Stained smears were made of the sediment retained on the surface of the filter. In several cases spores of *Bacillus larvae* were observed in stained smears of the sediment filtered out of honey known to have a large spore content. However, with honey containing fewer spores it was found impossible to concentrate them on a small enough area of filter in sufficient

numbers to recover and identify them under the microscope. Even with a comparatively large filtering surface, the process was so slow that the diluted honey would frequently start to ferment before it had all passed through the filter. A filter of smaller area would become clogged, preventing the passage of a sufficient quantity of honey.

Several unsuccessful attempts were made to recover spores of *Bacillus larvae* from honey by centrifuging samples diluted with an equal quantity of water. After considerable experimentation with honey of known spore content, it was found that it was necessary to dilute the honey to a much greater extent—1 part to at least 9 of water—in order to throw the spores down with the sediment. Apparently the specific gravity of these spores is so low that on centrifuging they remain in suspension in only slightly diluted honey.

The procedure finally used for demonstrating the presence of spores of *Bacillus larvae* in honey is as follows: Five c c of warmed honey is thoroughly mixed with 45 c c of distilled water in a 50 c c cone-shaped centrifuge tube made of heat-resistant glass. Duplicate quantities of each sample of honey are made up for examination. The diluted honey is then centrifuged at 2,000 revolutions per minute for one-half hour. Because of the difficulty of obtaining a satisfactory stained smear from the sediment thrown down in the presence of the sugars of the honey solution, all but 2 c c of the solution in each centrifuge tube is drawn off by means of a 50 c c pipette. Another 45 c c of distilled water is added, the sediment is thoroughly shaken up in the water, and the tubes are centrifuged again for 20 minutes. After all but 2 c c or less of the wash water has been removed, 0.01 c c of the sediment is removed by means of a capillary pipette and smeared on a cover glass over a surface of 1 cm², a small loopful of carbol fuchsin being mixed with the material before it is allowed to dry. After drying by gentle heat, the cover glass is mounted on a slide by means of a drop of distilled water and the smear is examined with an oil-immersion objective. Spores of *B. larvae* are identified by their size and shape in conjunction with their distinctive habit of breaking loose from the stained mass of the smear and of showing a delicate Brownian movement in the thin film of water between the two pieces of glass. In a few samples only one or two spores were seen in numerous fields examined or the spores did not have the typical appearance of spores of *B. larvae*. In such cases another test, in which twice as much honey was used, was made from the sample.

OBSERVATIONS

One hundred and ninety-one samples of honey were examined by this method. (Table 7.) Of these, 187 were regular commercial samples purchased in the open market and 2 were from the experimental apiary at Laramie. The other two were miscellaneous samples, one of which was obtained from a brood comb from a diseased colony and the other from a cappings melter which had been used with combs from an infected apiary.

TABLE 7.—Results of the examination of samples of honey for the presence of spores of *Bacillus larvae*

Source	Samples tested	Samples showing positive presence of spores resembling <i>Bacillus larvae</i>	Samples showing no evidence of spores *
Commercial samples from 30 States.....	187	15	172
Experimental.....	2	2	2
Miscellaneous.....	2	2	2
Total.....	191	17	174

* 29 of these samples were doubtful on the first examination, but repeated examinations gave negative results in each case.

Of the 187 samples of commercial honey obtained from 30 different States or Territories, 15, or 8 per cent, showed the presence of a sufficient number of spores resembling spores of *Bacillus larvae* to be designated as positive. In 29 of the commercial samples, or 15.5 per cent, one or two doubtful spores were seen in each case, but on repeated examinations none of these samples could be considered positive. Two of the four miscellaneous samples from infected sources were also found to contain spores of *B. larvae*.

Five of the samples showing the presence of spores of *Bacillus larvae* were fed to healthy 5-frame colonies during the summer of 1930. These samples consisted of from a pint to a quart of honey. No evidence of American foulbrood appeared in any of the five colonies during the entire brood-rearing season.

In order to determine the approximate number of spores in the samples of honey in which the presence of *Bacillus larvae* was demonstrated, a series of dilutions of spores was prepared as described for the work with cultures. A stained smear was made of 0.01 c c of each dilution spread over a 1-cm² surface of cover glass mounted with water and examined with the oil-immersion objective. By this means a definitely recognizable number of spores could be found down to the dilution of 2,000,000 spores per cubic centimeter, with a few single spores seen in occasional fields down to the dilution of 500,000 spores per cubic centimeter. (Table 8.) Then 1 c c of each dilution was added to 5 c c of distilled water in 15 c c centrifuge tubes and centrifuged at 2,000 revolutions per minute for 20 minutes. A stained smear made from 0.01 c c of each sediment showed a definitely recognizable number of spores down to the 5,000-spore dilution, with one or two doubtful spores in several fields from the 500-spore dilution. The sample containing the 50,000-spore dilution, which would be comparable to the sugar sirup containing the minimum number of spores per cubic centimeter fed to colonies in the spore-feeding experiments that produced infection, showed a great many more spores in each field examined by this method than did the sample of commercial honey that showed the greatest number of spores. Therefore, until a better quantitative method is devised, it seems reasonable to believe, from the indications of the preliminary work on this problem, that, even though the presence of a few spores of *B. larvae* may be

demonstrated in 5 c c quantities from a comparatively small percentage of samples of commercial honey, the numbers are far below the minimum necessary to produce infection when such honey is used in healthy colonies of bees. Before definite conclusions can be drawn, it will be desirable to examine many more samples of commercial honey and to feed to healthy colonies samples of honey in which the presence of spores has been demonstrated.

TABLE 8.—Microscopic examination of dilutions for spores of *Bacillus larvae* *

Number of spores per cubic centimeter in each dilution	Direct examination of 0.01 cubic centimeter	Examination of sediment after centrifuging 1 cubic centimeter	Number of spores per cubic centimeter in each dilution	Direct examination of 0.01 cubic centimeter	Examination of sediment after centrifuging 1 cubic centimeter	Number of spores per cubic centimeter in each dilution	Direct examination of 0.01 cubic centimeter	Examination of sediment after centrifuging 1 cubic centimeter
5,000,000,000	+	+	10,000,000	+	+	100,000	—	+
4,000,000,000	+	+	9,000,000	+	+	90,000	—	+
3,000,000,000	+	+	8,000,000	+	+	80,000	—	+
2,000,000,000	+	+	7,000,000	+	+	70,000	—	+
1,000,000,000	+	+	6,000,000	+	+	60,000	—	+
500,000,000	+	+	5,000,000	+	+	50,000	—	+
400,000,000	+	+	4,000,000	+	+	40,000	—	+
300,000,000	+	+	3,000,000	+	+	30,000	—	+
200,000,000	+	+	2,000,000	+	+	20,000	—	+
100,000,000	+	+	1,000,000	±?	+	10,000	—	+
90,000,000	+	+	800,000	—	+	9,000	—	—
80,000,000	+	+	700,000	—	+	8,000	—	—
70,000,000	+	+	600,000	—	+	7,000	—	—
60,000,000	+	+	500,000	±?	+	6,000	—	—
50,000,000	+	+	400,000	—	+	5,000	—	—
40,000,000	+	+	300,000	—	+	4,000	—	—
30,000,000	+	+	200,000	—	+	3,000	—	—
20,000,000	+	+				2,000	—	—
						1,000	—	—
						500	—	±
						50	—	—?
						5	—	—

* + Indicates that spores were found; — Indicates that spores were not found, by microscopic examination; ± indicates that the result was doubtful; ±? indicates that the positive was more doubtful than the negative; —? indicates that the absence of spores was not definite.

SUMMARY AND CONCLUSIONS

As a result of five years' study it has been found that, in order to produce American foulbrood infection in a healthy colony of bees, the sugar sirup used for inoculation must contain a certain initial number of spores of *Bacillus larvae*. Seventy-three colonies were inoculated during this time with numbers of spores ranging from approximately 5,000,000,000 to 100,000 per colony; 30 of these colonies receiving 50,000,000 spores or less. Of these 30 colonies, 2 out of 11 receiving 50,000,000 spores showed infection, but no colony receiving less than that number of spores developed disease. Therefore, the minimum infectious dose of *B. larvae* for a colony of bees seems to be approximately 50,000,000 spores in 1 liter of sugar sirup.

Preliminary experiments in which individual bee larvae were given known numbers of spores of *Bacillus larvae* in 0.01 c c quantities of sugar sirup show that infection can be produced by this method, but with considerable difficulty. From 50 to 100 larvae were inoculated with each dilution of spores, ranging in number from approximately 50,000,000 spores to, theoretically, 1 spore per larva. The minimum infectious dose was found to be 10,000,000 spores per larva fed in 0.01 c c of sugar sirup. These results indicate that the

minimum dose of spores of *B. larvae* that will produce American foulbrood infection must be large.

The germination of spores of *Bacillus larvae* and vegetative growth on a suitable artificial culture medium resulting from the inoculation of 556 culture tubes with seedings varying from approximately 50,000,000,000 to 500 spores per culture also shows that a certain minimum initial number of spores in the inoculum is necessary in order to produce growth. This minimum number of spores producing vegetative growth on a medium consisting of yeast-carrot extract, egg-yolk suspension, and agar was found to be approximately 50,000 in 1 c c of suspension inoculated.

The production of nitrite in this medium by the vegetative growth of *Bacillus larvae* serves as a fairly delicate and reliable indicator of such growth.

There was a tendency for the seedings containing the smaller numbers of spores of *Bacillus larvae* to require a longer period of incubation than the larger seedings in order to produce vegetative growth. However, there was a considerable variation in the germination time of many of the seedings of spores, in one case a seeding of 9,000,000 spores requiring 27 days' incubation to produce growth and another of 70,000 spores requiring only 6 days. This variation, thought to be due to the variable character known as dormancy in bacterial spores, prevented more than a slight correlation.

In the group of cultures comprising seedings between 5,000,000,000 and 9,000,000 spores, only 1.67 per cent required more than 10 days' incubation to produce vegetative growth, 100 per cent having shown growth after 30 days. In the group of cultures comprising seedings between 8,000,000 and 500,000 spores, 71.93 per cent required more than 10 days' incubation, while 53.81 per cent showed no growth at the end of 30 days' incubation. In the group of cultures comprising seedings between 400,000 and 50,000 spores, 97.18 per cent required more than 10 days' incubation, while 91.55 per cent of the group showed no growth at the end of 30 days. Below 50,000 spores no growth was obtained. In other words, below a seeding of 9,000,000 spores an increasing number of the smaller spore seedings required a longer period of incubation. About 80 per cent of all the positive cultures were obtained during the first 10 days of incubation, although this was approximately only 30 per cent of all the cultures made; at the end of 30 days' incubation only about 38 per cent of all the cultures had shown any growth.

It was found possible to demonstrate the presence of spores of *Bacillus larvae* in 15 out of 187, or in 8 per cent, of the samples of commercial honey examined by means of the centrifuge and the microscope. The preliminary results indicate that, even though spores of *B. larvae* may be demonstrated in a certain percentage of samples of commercial honey, in most instances they are probably present in such small numbers as to be less than the minimum number, 50,000,000 per liter, found to be capable of producing disease, and therefore are ineffective in the spread of American foulbrood.

LITERATURE CITED

- (1) AHRENS, H. G.
1930. NEW FACTS ABOUT FORMALIN TREATMENT. *Amer. Bee Jour.* 70: 61-62.
- (2) BREED, R. S., AND BREW, J. D.
1916. COUNTING BACTERIA BY MEANS OF THE MICROSCOPE. *N. Y. State Agr. Expt. Sta. Tech. Bul.* 49, 31 p., illus.
- (3) BUCHANAN, R. E.
1918. LIFE PHASES IN A BACTERIAL CULTURE. *Jour. Infect. Diseases* 23:109-125, illus.
- (4) BURKE, G. S.
1923. STUDIES ON THE THERMAL DEATH TIME OF SPORES OF CLOSTRIDIUM BOTULINUM. 2. THE DIFFERENTIAL STAINING OF LIVING AND DEAD SPORES. *Jour. Infect. Diseases* 32:[433]-438, illus.
- (5) ———
1923. STUDIES ON THE THERMAL DEATH TIME OF SPORES OF CLOSTRIDIUM BOTULINUM. 3. DORMANCY OR SLOW GERMINATION OF SPORES UNDER OPTIMUM GROWTH CONDITIONS. *Jour. Infect. Diseases* 33:[274]-284.
- (6) BURKE, V., SPRAGUE, A., AND BARNER, LA V.
1925. DORMANCY IN BACTERIA. *Jour. Infect. Diseases* 36:[555]-560.
- (7) BURNSIDE, C. E.
1931. DISINFECTION OF AMERICAN FOULBROOD COMBS BY FUMIGATION BY FORMALDEHYDE. *Bee World* 12:3-7, 16-19.
- (8) CORKINS, C. L.
1928. QUARTERLY REPORT. *Wyo. Beeline* 5:25-26.
- (9) ELDFORD, W. J.
1928. ULTRAFILTRATION. (AN HISTORICAL SURVEY, WITH SOME REMARKS ON MEMBRANE PREPARATION TECHNIQUE). *Jour. Roy. Micros. Soc.* (3) 48:36-45, illus.
- (10) FRACKER, S. B.
1925. ARE COMMERCIAL HONEY SHIPMENTS LARGELY RESPONSIBLE FOR THE DISSEMINATION OF AMERICAN FOULBROOD? *Jour. Econ. Ent.* 18:372-380.
- (11) GATES, F. L.
1920. A METHOD OF STANDARDIZING BACTERIAL SUSPENSIONS. *Jour. Expt. Med.* 31:105-114, illus.
- (12) HENRICI, A. T.
1928. MORPHOLOGIC VARIATION AND THE RATE OF GROWTH OF BACTERIA. 194 p., illus., Springfield, Ill., and Baltimore, Md. (Monographs on Agricultural and Industrial Microbiology, v. 1.)
- (13) JORDAN, E. O., AND FALK, I. S., editors.
1928. THE NEWER KNOWLEDGE OF BACTERIOLOGY AND IMMUNOLOGY. 1196 p., illus. Chicago.
- (14) KELLEY, T. L.
1923. STATISTICAL METHOD. 390 p., illus. New York.
- (15) KOSER, S. A., AND MILLS, J. H.
1925. DIFFERENTIAL STAINING OF LIVING AND DEAD BACTERIAL SPORES. *Jour. Bact.* 10:25-36.
- (16) LINEBURG, B.
1925. STRAIN OF IMMUNE BEES. *Gleanings Bee Cult.* 53:709-710.
- (17) LOCHHEAD, A. G.
[1927.] FURTHER STUDIES OF BACILLUS LARVÆ, THE CAUSE OF AMERICAN FOULBROOD OF BEES. *Canada Expt. Farms, Div. Bact. Rpt.* 1926:13-16.
- (18) ———
1928. CULTURAL STUDIES OF BACILLUS LARVÆ (WHITE). *Sci. Agr.* 9: 80-89, illus.
- (19) ——— AND HERON, D. A.
1929. MICROBIOLOGICAL STUDIES OF HONEY. I. HONEY FERMENTATION AND ITS CAUSE. II. INFECTION OF HONEY BY SUGAR-TOLERANT YEASTS. *Canada Dept. Agr. Bul.* (n. s.) 116, 47 p., illus.
- (20) MCCRAY, A. H., AND WHITE, G. F.
1918. THE DIAGNOSIS OF BEE DISEASES BY LABORATORY METHODS. *U. S. Dept. Agr. Bul.* 671, 15 p., illus.

- (21) MARVIN, G. E.
1928. THE OCCURENCE AND CHARACTERISTICS OF CERTAIN YEASTS FOUND IN FERMENTED HONEY. *Jour. Econ. Ent.* 21:363-370, illus.
- (22) MERRILL, J. H.
1927. AN INITIAL OUTBREAK OF FOULBROOD. *Amer. Bee Jour.* 67:414-415.
- (23) MILLEN, F. E.
1928. SPREADING FOULBROOD. *Beekeeper* 36:134.
- (24) MORRISON, E. W., AND RETTGER, L. F.
1930. BACTERIAL SPORES. II. A STUDY OF BACTERIAL SPORE GERMINATION IN RELATION TO ENVIRONMENT. *Jour. Bact.* 20:313-342.
- (25) ROBERTSON, T. B.
1923. THE CHEMICAL BASIS OF GROWTH AND SENESCENCE. 389 p., illus. Philadelphia and London. [Original not seen.]
- (26) STURTEVANT, A. P.
1924. THE DEVELOPMENT OF AMERICAN FOULBROOD IN RELATION TO THE METABOLISM OF ITS CAUSATIVE ORGANISM. *Jour. Agr. Research* 28:129-168, illus.
- (27) ————
1930. PRELIMINARY REPORT CONCERNING FACTORS RELATED TO CERTAIN OF THE GROWTH PHASES OF BACILLUS LARVAE. *Jour. Econ. Ent.* 23:453-459.
- (28) SWANN, M. B. R.
1924. ON THE GERMINATION PERIOD AND MORTALITY OF THE SPORES OF BACILLUS ANTHRACIS. *Jour. Path. and Bact.* 27:130-134.
- (29) TOUMANOFF, K.
1929. NOTE SUR L'INFECTION DES LARVES D'ABEILLES PAR BACILLUS LARVÆ. *Bul. Acad. Vét. France* 2:45-49.
- (30) WHITE, G. F.
1920. AMERICAN FOULBROOD. U. S. Dept. Agr. Bul. 809, 46 p., illus.
- (31) ZINSSER, H.
1927. A TEXTBOOK OF BACTERIOLOGY; A TREATISE ON THE APPLICATION OF BACTERIOLOGY AND IMMUNOLOGY TO THE ETIOLOGY, DIAGNOSIS, SPECIAL THERAPY AND PREVENTION OF INFECTIOUS DISEASES, FOR STUDENTS AND PRACTITIONERS OF MEDICINE AND PUBLIC HEALTH . . . Rewritten, rev. and reset . . . Ed. 6, 1053 p., illus. New York and London.

HETEROTHALLISM AND HYBRIDIZATION IN SPHACELOTHECA SORGHII AND S. CRUENTA¹

By H. A. RODENHISER²

Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry,
United States Department of Agriculture

INTRODUCTION

It has been shown by a number of investigators that certain of the smut fungi are heterothallic. This means that in such fungi the fusion of two hyphal or monosporidial lines of opposite sex is necessary before normal infection and the subsequent production of chlamydospores can take place. Kniep (14)³ in 1919, working with *Ustilago violaceae* (Pers.) Fckl., was the first to show that fusion takes place only between certain sporidia. Evidence of heterothallism in other smut fungi was later reported by Bauch (1), Stakman and Christensen (16), Dickinson (5), Hanna and Popp (12), and Flor (9). Fusion between monosporidial lines of opposite sex of different species of smut fungi has been observed. Kniep (15), working with several species of *Ustilago*, observed fusion between monosporidial lines of different species. Dickinson (6) was unable to obtain infection of oat seedlings with monosporidial lines of *U. levis* (Kell. and Sw.) Magn. and *U. hordei* (Pers.) Kell. and Sw., but when sporidia of one sex of *U. hordei* were combined with those of opposite sex of *U. levis*, infection resulted. Apparently his study was not carried far enough to determine whether hybrid chlamydospores could be produced as a result of the cross. Hanna and Popp (12) and Holton (13) obtained hybrid chlamydospores as a result of interspecific crosses made between *U. levis* and *U. avenae*. The hybrid chlamydospores were found to be echinulate, while chlamydospores from inbred lines of each species had markings characteristic of the respective species. Flor (9) recently found *Tilletia levis* Kühn and *T. tritici* (Bjerk.) Wint. to be heterothallic. Hybrid chlamydospores were obtained as a result of crosses between physiologic forms of a single species and of crosses between the two species. The chlamydospores produced by the species cross were identical in appearance with those of *T. levis*.

The fact that certain of the smut fungi have been found to be heterothallic and that hybrid chlamydospores may be produced as a result

¹ Received for publication Mar. 25, 1932; issued September, 1932.

² The writer wishes to express his appreciation to A. G. Johnson, principal pathologist, Division of Cereal Crops and Diseases, for assistance in the preparation of the manuscript. He is indebted also to Prof. L. E. Melchers, of the Kansas State Agricultural College, for chlamydospore material of the three physiologic forms of *Sphacelotheca sorghii* and the two collections of *S. cruenta*.

³ Reference is made by number (italic) to Literature Cited, p. 295.

of intraspecific and interspecific crosses suggests strongly that new physiologic forms may arise as a result of hybridization. In a study of the origin of pathogenically different strains of *Sphacelotheca sorghi* (Lk.) Clint., the present investigations were made to determine whether *S. sorghi* and *S. cruenta* (Kühn) Potter are heterothallic and to what extent hybridization may take place between physiologic forms and between the two species.

MATERIALS AND METHODS

Single sporidial lines used in these tests were isolated from chlamydospores of three pathogenically distinct physiologic forms of *Sphacelotheca sorghi*, namely, the common, milo, and feterita strains as identified by Tisdale, Melchers, and Clemmer (17), and from two collections of *S. cruenta*.⁴ The method used in isolating single sporidia was similar to that described by Dickinson (4) and Hanna (10), except that the isolating needle was attached to a Chambers micro-manipulator. Single chlamydospores taken from a single sorus were placed on hanging drops of 2 per cent potato-dextrose agar, and when they had germinated the sporidia were removed from the promycelia and propagated separately. Subcultures of these lines, to be used as inoculum, were prepared by growing each line separately in a 4 per cent carrot extract. The cultures were allowed to develop three weeks before being used to inoculate sorghum plants.

Reed kafir sorghum (C. I.⁵ 628), which is susceptible to all known collections of both *Sphacelotheca sorghi* and *S. cruenta*, was used in all the experiments. When the plants were 5 weeks old they were inoculated with the sporidial suspensions. By means of a hypodermic syringe the inoculum was injected into the plants as near the growing point as possible. When a combination of two monosporidial lines was used the two cultures were mixed just before the inoculation. Controls were inoculated in the same manner with sterile carrot extract. The plants were grown singly in pots in the greenhouse until six days after they were inoculated, and then transplanted to the field plots at the Arlington Experiment Farm, Rosslyn, Va. (near Washington, D. C.). Ten plants were used for each inoculation. In some of the tests, as indicated later, the plants were grown to maturity in the greenhouse.

The common, milo, and feterita strains of *Sphacelotheca sorghi* are referred to in this paper as forms S1, S2, and S3, respectively, and collections of *S. cruenta* as C1 and C2. Where different chlamydospores of a particular form or collection were used they are distinguished by a letter. In most cases a record was kept of the position on the promycelium occupied by each sporidium, and for convenience the sporidia are numbered 1, 2, 3, and 4, from the tip of the promycelium to the chlamydospore. Thus, S1B3 would refer to a sporidium isolated from the third segment of the promycelium from a particular chlamydospore B of form 1, *S. sorghi*.

⁴ The collections of *Sphacelotheca cruenta* referred to in this paper as C1 and C2 were received from Professor Melchers as Reed's and Ficke's collections, respectively.

⁵ C. I. denotes accession number of Division of Cereal Crops and Diseases, formerly Office of Cereal Investigations.

HETEROTHALLISM

SPHACELOTHECA SORGHI

Each of the four first-formed sporidia from each of three chlamydospores of *Sphacelotheca sorghi*, form 1, was isolated and cultured as previously described. Subcultures of these monosporidial lines, alone and in all possible paired combinations for each chlamydospore, were then used to inoculate sorghum plants. The results of these inoculations are presented in Figure 1. The plus sign (+) indicates that the inoculated plants developed smutty heads, and the minus sign (−) indicates that no smut developed.

It is evident from the results obtained that *Sphacelotheca sorghi* is heterothallic, since none of the 12 monosporidial lines when injected alone caused infection, whereas plants inoculated with certain paired combinations or sex groups developed smutty heads. Bauch (2) found that *Ustilago longissima* (Sow.) Tul. might have sporidia belonging to many sexual groups. Hanna (11) and Christensen (3) showed that at least four sexual groups might be present in sporidia

	1	2	3	4
1	−	+	−	+
2	+	−	+	−
3	−	+	−	+
4	+	−	+	−

S1A

	1	2	3	4
1	−	+	+	+
2	+	−	−	−
3	+	−	−	−
4	+	−	−	−

S1B

	1	2	3	4
1	−	+	+	−
2	+	−	−	+
3	+	−	−	+
4	−	+	+	−

S1C

FIGURE 1.—Results of inoculating sorghum plants with monosporidial lines of *Sphacelotheca sorghi* used singly and in pairs, from each of the chlamydospores S1A, S1B, and S1C.

of *Ustilago zeae* (Beckm.) Ung. In the present investigations with *S. sorghi*, sporidia belonging to only two sexual groups have been isolated. However, with reference to sex the sporidia were arranged on the promycelium in three different positions. From chlamydospore S1A, sporidia 1 and 3 were of the opposite sex from that of sporidia 2 and 4; while from chlamydospore S1C, sporidia 1 and 4 were of the opposite sex from that of sporidia 2 and 3. Two sexual groups were present in sporidia from chlamydospore S1B, but they were not in pairs, sporidium 1 being of the opposite sex from that of sporidia 2, 3, and 4.

Christensen (3), working with *Ustilago zeae*, found that when the monosporidial lines isolated from a promycelium are paired in all possible combinations they may fall into two sexual groups, but that when paired with sporidia from another promycelium they frequently belong to three or four distinct sexual groups. In the present investigation all possible paired combinations were made between the four monosporidial lines from chlamydospores S1A and S1B. The

results of these matings (fig. 2) indicate that the sex groups reacted normally and that no evidence was obtained of complete interfertility or intersterility. In greenhouse experiments similar results were obtained from several matings between sporidia of known sex of physiologic forms S1, S2, and S3.

		S1A				S1B			
		1	2	3	4	1	2	3	4
S1A	1	-	+	-	+	-	+	+	+
	2	+	-	+	-	+	-	-	-
	3	-	+	-	+	-	+	+	+
	4	+	-	+	-	+	-	-	-
S1B	1	-	+	-	+	-	+	+	+
	2	+	-	+	-	+	-	-	-
	3	+	-	+	-	+	-	-	-
	4	+	-	+	-	+	-	-	-

FIGURE 2.—Results of inoculating sorghum plants with four monosporidial lines of *Sphacelotheca sorghi* from chlamydospore S1A paired with the four monosporidial lines from chlamydospore S1B

SPHACELOTHECA CRUENTA

Each of the four first-formed sporidia from each of three chlamydospores of collection C1 of *Sphacelotheca cruenta* was isolated, cultured, and used in inoculations. The results of inoculating sorghum plants with these monosporidial lines alone and in paired combinations for each chlamydospore are recorded in Figure 3.

	1	2	3	4
1	-	+	+	-
2	+	-	-	+
3	+	-	-	+
4	-	+	+	-

C1A

	1	2	3	4
1	-	-	-	+
2	-	-	-	+
3	-	-	-	+
4	+	+	+	-

C1B

	1	2	3	4
1	-	+	+	+
2	+	-	-	-
3	+	-	-	-
4	+	-	-	-

C1C

FIGURE 3.—Results of inoculating sorghum plants with monosporidial lines of *Sphacelotheca cruenta* used singly and in pairs, from each of the chlamydospores C1A, C1B, and C1C

These data indicate that *Sphacelotheca cruenta* is heterothallic and that the sporidia from each promycelium belong to two sexual groups. The two groups from chlamydospore C1A were so arranged that sporidia 1 and 4 were of the opposite sex from that of sporidia 2 and 3. Chlamydospores C1B and C1C each produced three sporidia of one

sexual group and one of the opposite group. However, the arrangement differed in that the sporidia of C1B were grouped so that sporidia 1, 2, and 3 were of the opposite sex from that of sporidium 4 and those of C1C so that sporidium 1 was of the opposite sex from that of sporidia 2, 3, and 4.

A test was also made with *Sphacelotheca cruenta* to determine the sex relationship between sporidia of two different chlamydo-spores. The sporidia of chlamydo-spore C1A were paired in all possible combinations with those of chlamydo-spore C1B, and the results obtained are recorded in Figure 4. It is evident from these data that the sex groups reacted normally, as they did in the outbred lines of *S. sorghi*, and also that there is no indication of complete interfertility or inter-sterility.

From the evidence just presented it appears that in both *Sphacelotheca sorghi* and *S. cruenta* the factors determining sex may segre-

		C1A				C1B			
		1	2	3	4	1	2	3	4
C1A	1	-	+	+	-	+	+	+	-
	2	+	-	-	+	-	-	-	+
	3	+	-	-	+	-	-	-	+
	4	-	+	+	-	+	+	+	-
C1B	1	+	-	-	+	-	-	-	+
	2	+	-	-	+	-	-	-	+
	3	+	-	-	+	-	-	-	+
	4	-	+	+	-	+	+	+	-

FIGURE 4.—Results of inoculating sorghum plants with four monosporidial lines of *Sphacelotheca cruenta* from chlamydo-spore C1A paired with the four monosporidial lines from chlamydo-spore C1B

gate on a 2:2 or 1:3 basis. The sex factors of chlamydo-spores S1A, S1C, and C1A all segregated on a 2:2 basis, and from the arrangement of the sex groups on the promycelium it is evident that reduction of factors for sex took place in the second division of the fusion nucleus. Factors determining sex in chlamydo-spores S1B, C1B, and C1C segregated on a 1:3 basis. Segregation in this ratio is more difficult to explain. It may have taken place as a result of segregation in the third division of the nucleus or of some abnormal type of segregation. Hanna (11) and Christensen (3), working with *Ustilago zeae*, and Dickinson (7), working with *U. levis*, found similar segregation ratios for sex. These investigators found also that the segregation of sex factors may be independent of the segregation of factors for cultural characteristics. In the present investigation cultural comparisons of the four monosporidial lines from each of the three chlamydo-spores of *S. sorghi* and of *S. cruenta* were made on 2.5 per cent potato-dextrose

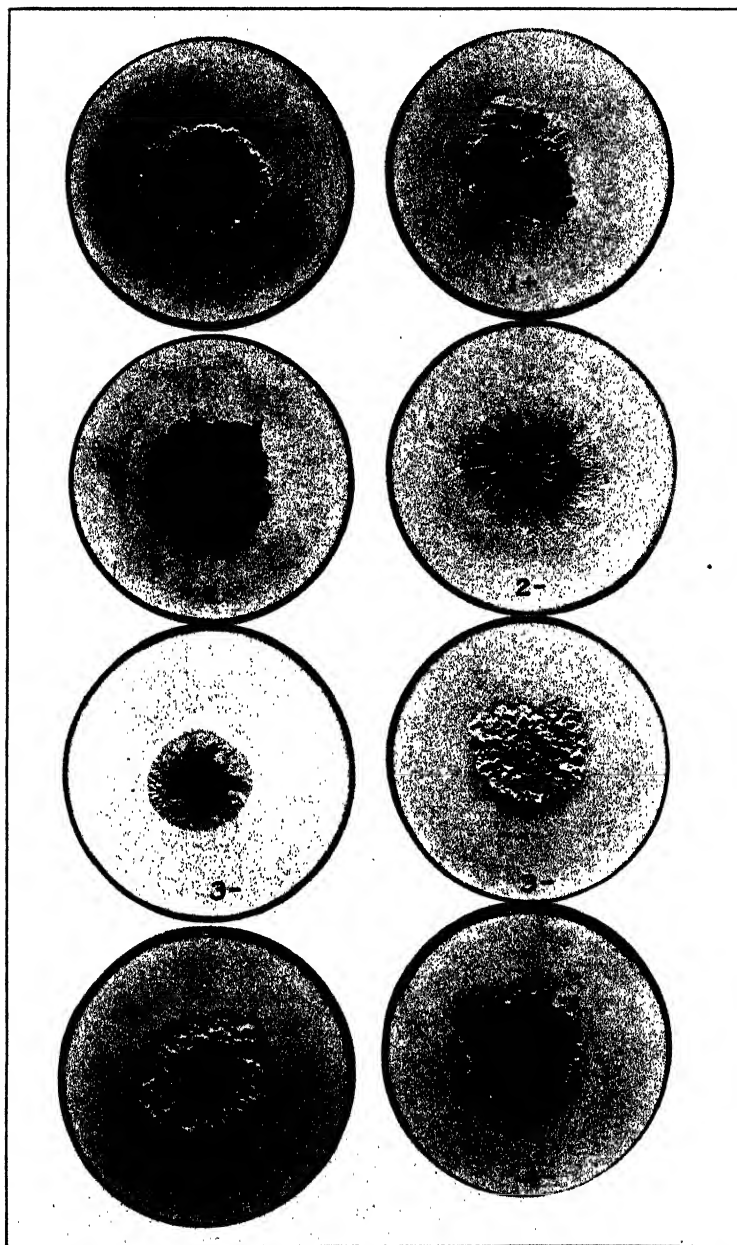
agar. The striking differences in the cultural characteristics of monosporidial lines from chlamydospores S1B and S1C (*S. sorghi*) may be seen in Plate 1. Similar differences in lines from chlamydospore C1C (*S. cruenta*) may be seen in Plate 2. It was evident from these tests that the factors determining sex in sporidia of both *S. sorghi* and *S. cruenta* segregated independently of those determining cultural characteristics. For example, the segregation ratio of factors for sex in chlamydospores S1A, S1B, and S1C of *S. sorghi* were 2:2, 1:3, and 2:2, respectively, while the factors determining color of the cultures of monosporidial lines from all these chlamydospores segregated on a 1:1:1:1 basis. In chlamydospores C1A, C1B, and C1C of *S. cruenta* the sex segregation ratios were 2:2, 1:3, and 1:3, respectively, as compared with the segregation ratios for color of colonies of 4:0 from chlamydospores C1A and 1:1:1:1 in lines from chlamydospores C1B and C1C. In these tests it was evident also that the factors determining other cultural characteristics such as topography, surface, consistency, rate of growth, and margin of colony segregated independently of those determining sex.

The ability of paired lines to produce chlamydospores in the plant has been considered in this paper as the criterion of sex compatibility. If two lines did not produce chlamydospores they were considered to be of the same sex. Some evidence was obtained that sex compatibility or incompatibility could be detected within a few days after the plants had been inoculated with the paired lines. It was found that on the leaves of plants inoculated with certain paired monosporidial lines of *Sphacelotheca sorghi* or *S. cruenta* small but distinct chlorotic areas developed in four to six days after inoculation, whereas the leaves of plants inoculated with certain other matings or with a monosporidial line remained normal. (Pl. 3.)

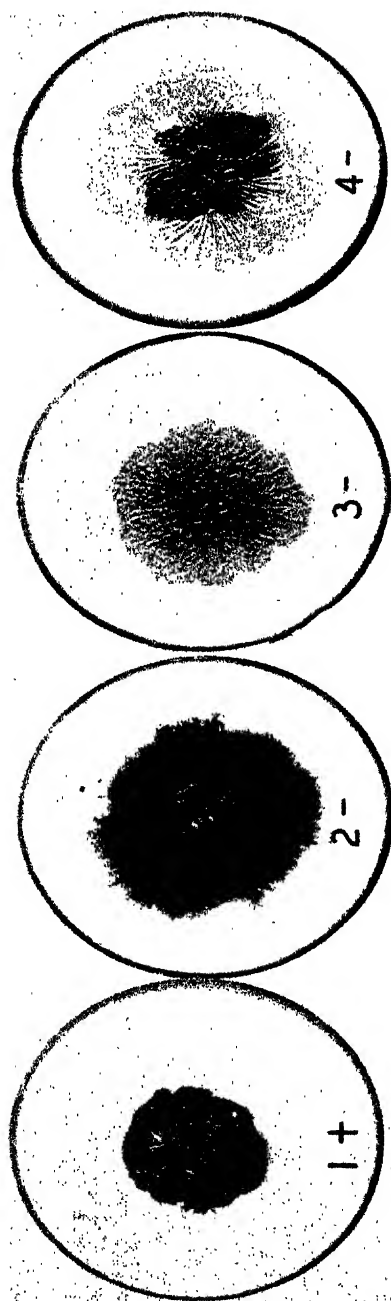
Under the conditions of these experiments nearly all the chlorotic spots disappeared within a week or 10 days after they first developed. However, on a few leaves of plants that had been inoculated with paired lines of *Sphacelotheca sorghi* the spots did not disappear and galls about the size of a beet seed developed, within which mature chlamydospores were formed. These chlamydospores were found to germinate normally. When all the plants had matured it was found that smutty heads developed only on those plants on whose leaves chlorotic spots had previously formed. In only one case did a paired line that caused flecking of the leaves fail to produce smut in the head. From these data it appears that the formation of the chlorotic spots is an early indication of sex compatibility, the factor or factors for which are closely associated if not linked with those for pathogenicity.

HYBRIDIZATION

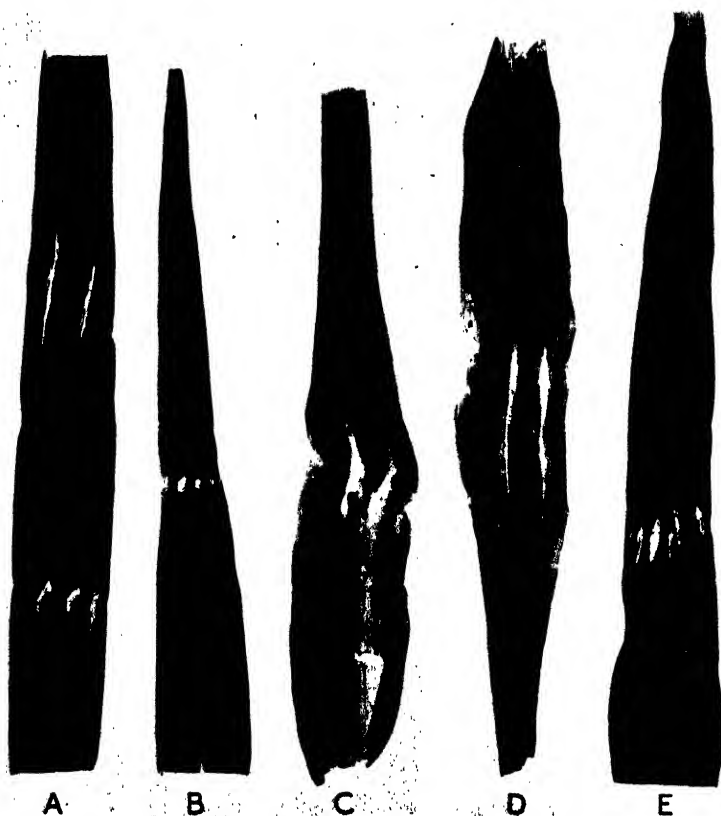
The four monosporidial lines isolated from chlamydospore S1A (*Sphacelotheca sorghi*) were paired in all possible combinations with those from chlamydospore C1A (*S. cruenta*). Reed kafir plants were inoculated with these combinations, and the results are recorded in Figure 5. It is clear from these data that *S. sorghi* and *S. cruenta* hybridize readily, as shown by the production of chlamydospores in the host. Furthermore, the sex groups as identified in the experiment on heterothallism reacted normally in the interspecific crosses. Similar results were obtained in greenhouse tests with a monosporidial



Cultures of monosporidial lines from two chlamydospores (S1B and S1C) of *Sphacelotheca sorghi* on potato-dextrose agar. The four cultures at left are from chlamydospore S1C; those at right are from chlamydospore S1B. The cultures are numbered 1, 2, 3, and 4, according to the position of the sporidia, from the tip of the promycellium to the chlamydospore. The sex is indicated by a plus (+) or minus (-) sign, according to the pairing reactions shown in Figure 1



Cultures of the four monosporidial lines from chlamydospore C1C of *Sphacelotheca cruenta* on potato-dextrose agar. The cultures are numbered, according to the position of the sporidia, from the tip of the promycelium to the chlamydospore. The sex is indicated by a plus (+) or (-) sign, according to the pairing reactions shown in Figure 3



Sorghum leaves from plants inoculated with single or monosporidial paired lines of *Sphacelotheca sorghi* (A-C) and *S. cruenta* (D, E) as follows: A, Inoculated with monosporidial line S1A1 (no mottling); B, inoculated with monosporidial line S1A2 (no mottling); C, inoculated with sexually compatible lines S1A1 and S1A2 (note mottling); D, inoculated with sexually compatible lines C1A1 and C1B1 (note mottling); E, inoculated with sexually incompatible lines C1A1 and C1B4 (no mottling)

line of C1A paired with monosporidial lines of two additional physiologic forms of *S. sorghi*, namely, S2 and S3.

Apparently the sexual compatibility between interspecific lines also can be determined soon after the plants are inoculated. Certain combinations caused flecking of the plant leaves, as did the sexually compatible pairs of intraspecific lines, and it is significant that only those plants inoculated with the combination that caused flecking of the leaves produced smutty heads. As stated previously, leaf galls occasionally formed on plants inoculated with *Sphacelotheca sorghi*, but in these tests no galls developed as a result of inoculations with interspecific crosses.

Holton (13) found that hybrid chlamydospores produced as a result of a cross between *Ustilago levis* and *U. avenae* germinated normally, but that only an occasional primary sporidium developed when iso-

		S1A				C1A			
		1	2	3	4	1	2	3	4
S1A	1	-	+	-	+	+	-	-	+
	2	+	-	+	-	-	+	+	-
	3	-	+	-	+	+	-	-	+
	4	+	-	+	-	-	+	+	-
C1A	1	+	-	+	-	-	+	+	-
	2	-	+	-	+	+	-	-	+
	3	-	+	-	+	+	-	-	+
	4	+	-	+	-	-	+	+	-

FIGURE 5.—Results of inoculating sorghum plants with four monosporidial lines of *Sphacelotheca sorghi* from chlamydospore S1A paired with the four monosporidial lines of *S. cruenta* from chlamydospore C1A

lated. In the present investigations with *Sphacelotheca sorghi* and *S. cruenta* germination tests were made of a large number of primary sporidia isolated from germinating interspecific hybrid chlamydospores. The percentage of germination was found to be just as high as that of sporidia isolated from chlamydospores of inbred lines of either *S. sorghi* or *S. cruenta*. Furthermore, the hybrid chlamydospores were found to be pathogenic, since from 11 to 40 per cent of the Reed kafir plants inoculated with these hybrids developed smutty heads.

Sphacelotheca sorghi and *S. cruenta* have been distinguished macroscopically by the type of membrane surrounding the sori. This structure in the case of *S. sorghi* is relatively thick, grayish brown in color, and seldom ruptured except from mechanical injury. The outer membrane inclosing the sori of *S. cruenta* is thinner and more fragile and ruptures soon after the panicle has emerged, thereby exposing the mass of black chlamydospores. Microscopically, the two species have

been differentiated according to the type of sterile cells produced. Elongated hyaline cells of small diameter, which adhere in chains when the tissue is macerated, are supposed to be typical of *S. sorghi*. The sterile cells reported to be typical of *S. cruenta* are hyaline and spherical, having a diameter about twice that of the chlamydospores, and adhere rather loosely in clumps. In the present investigations, in intraspecific crosses the sori in the panicles were macroscopically characteristic of those of the respective species, whereas in the interspecific crosses sori of the type characteristic of *S. cruenta* were always dominant in the F_1 and the F_2 generations. Microscopic examination of sterile cells revealed the fact that both types of cells were present not only in the interspecific hybrids but also in the intraspecific crosses and in a number of macroscopically distinct specimens of both species. In some sori the sterile cells, which are supposed to be characteristic of the species, predominated; in others the distribution was more even, making it difficult to determine microscopically to which species the smut belonged. Tisdale, Melchers, and Clemmer (17) likewise found both types of sterile cells in mature sori of certain collections of the milo and hegari strains of *S. sorghi* and in a number of authentic specimens of both *S. sorghi* and *S. cruenta*. These results indicate that the method of differentiating the two species on the basis of the type of sterile cells produced is not entirely satisfactory.

CONCLUSIONS

From the data presented it is evident that both *Sphacelotheca sorghi* and *S. cruenta* are heterothallic. Monosporidial lines isolated from chlamydospores of the common, milo, and feterita strains of *S. sorghi* and from a collection of *S. cruenta* failed to cause infection of Reed kafir sorghum. When inoculated with sexually compatible lines this variety became infected, as evidenced by the production of chlamydospores in the host.

By mating a monosporidial line of a pathogenically distinct physiologic form of *Sphacelotheca sorghi* with a line of opposite sex of another physiologic form or of another species (*S. cruenta*) hybrid chlamydospores were formed in the host. The fact that these intraspecific and interspecific hybrid chlamydospores have been produced under controlled conditions strengthens the assumption that hybridization accounts, at least in part, for the occurrence of pathogenically distinct strains of the kernel smuts of sorghum. Theoretically these hybrids should contain factors for pathogenicity from both parents, and a recombination of these factors should develop new strains that may differ in virulence from either of the parents. Preliminary tests of the pathogenicity of these hybrids have been made, and the results indicate that the hybrids do differ from either of their parents in ability to infect certain varieties of sorghum.

Ficke and Johnston (8) reported that the common, milo, and feterita strains of *Sphacelotheca sorghi* may be distinguished by cultural characteristics on various kinds of nutrient media. In the present investigation, however, it was found that the cultural characteristics of four primary monosporidial lines isolated from a single promycelium of the common strain were distinctly different. In fact, from three chlamydospores isolated from a single smut sorus, 10 of the 12 primary sporidia isolated were distinctly different in type of growth on artificial

media. These results indicate that the common strain of *S. sorghi* can not be distinguished from other pathogenically distinct strains by any one particular type of growth on artificial media.

SUMMARY

Sphacelotheca sorghi (Lk.) Clint. and *S. cruenta* (Kühn) Potter are heterothallic. Monosporidial lines of both species failed to produce chlamydospores in the host plants, but when the plants were inoculated with two monosporidial lines of opposite sex smutted heads were produced.

Monosporidial lines from both *Sphacelotheca sorghi* and *S. cruenta* were found to belong to two sex groups. In intraspecific and interspecific crosses these sex groups were found to be constant and there was no evidence of complete intersterility or interfertility.

Factors determining sex of monosporidial lines of both species segregated on a 2:2 and 1:3 basis. The factors determining sex segregated independently of those determining type of growth of monosporidial lines on artificial media.

Some evidence was obtained indicating that the sexual compatibility of paired lines may be detected soon after the sorghum plants have been inoculated. On leaves of plants inoculated with sexually compatible paired lines distinct chlorotic areas developed four to six days after inoculation, whereas plants inoculated with monosporidial or sexually incompatible lines remained normal.

Sphacelotheca sorghi and *S. cruenta* are interfertile. Intraspecific crosses produced sori in the panicles that were macroscopically characteristic of the species, while interspecific crosses produced sori macroscopically characteristic of *S. cruenta*.

Sterile cells of both the elongated and the spherical type were found to be present in sori resulting from both intraspecific and interspecific crosses.

LITERATURE CITED

- (1) BAUCH, R.
1923. ÜBER USTILAGO LONGISSIMA UND IHRE VARIETÄT MACROSPORA. Ztschr. Bot. 15: [241]-279, illus.
- (2) ———
1930. ÜBER MULTIPOLARE SEXUALITÄT BEI USTILAGO LONGISSIMA. Arch. Protistenk. 70: 417-466, illus.
- (3) CHRISTENSEN, J. J.
1931. STUDIES ON GENETICS OF USTILAGO ZEAE. Phytopath. Ztschr. 4(2): 129-188.
- (4) DICKINSON, S.
1926. A METHOD OF ISOLATING AND HANDLING INDIVIDUAL SPORES AND BACTERIA. Roy. Soc. Med. [London], Proc. 19: 1-4, illus.
- (5) ———
1927. EXPERIMENTS ON THE PHYSIOLOGY AND GENETICS OF THE SMUT FUNGI. HYPHAL-FUSION. Roy. Soc. [London], Proc., Ser. B 101: 126-136, illus.
- (6) ———
1927. EXPERIMENTS ON THE PHYSIOLOGY AND GENETICS OF THE SMUT FUNGI. SEEDLING INFECTION. Roy. Soc. [London], Proc., Ser. B 102: 174-176.
- (7) ———
1931. EXPERIMENTS ON THE PHYSIOLOGY AND GENETICS OF THE SMUT FUNGI. CULTURAL CHARACTERS. PART II. THE EFFECT OF CERTAIN EXTERNAL CONDITIONS ON THEIR SEGREGATION. Roy. Soc. [London], Proc., Ser. B 108: 395-423.

-
- (8) FICKE, C. H., and JOHNSTON, C. O.
1930. CULTURAL CHARACTERISTICS OF PHYSIOLOGIC FORMS OF SPHACELOTHECA SORGHI. *Phytopathology* 20: 241-249, illus.
- (9) FLOB, H. H.
1932. HETEROTHALLISM AND HYBRIDIZATION IN TILLETIA TRITICI AND T. LEVIS. *Jour. Agr. Research* 44: 49-58.
- (10) HANNA, W. F.
1928. A SIMPLE APPARATUS FOR ISOLATING SINGLE SPORES. *Phytopathology* 18: 1017-1021, illus.
- (11) ———
1929. STUDIES IN THE PHYSIOLOGY AND CYTOLOGY OF USTILAGO ZEAE AND SOROSPORIUM REILIANUM. *Phytopathology* 19: 415-442, illus.
- (12) ——— and POPP, W.
1930. RELATIONSHIP OF THE OAT SMUTS. *Nature* [London] 126: 843-844.
- (13) HOLTON, C. S.
1931. HYBRIDIZATION AND SEGREGATION IN THE OAT SMUTS. *Phytopathology* 21: 835-842, illus.
- (14) KNIEP, H.
1919. UNTERSUCHUNGEN ÜBER DEN ANTHERENBRAND (USTILAGO VIO-LACEAE PERS.). EIN BEITRAG ZUM SEXUALITÄTSPROBLEM. *Ztschr. Bot.* 11: [257]-284.
- (15) ———
1926. ÜBER ARTKREUZUNGEN BEI BRANDPILZEN. *Ztschr. Pilzkunde* 5: [217]-247, illus.
- (16) STAKMAN, E. C., and CHRISTENSEN, J. J.
1927. HETEROTHALLISM IN USTILAGO ZEAE. *Phytopathology* 17: 827-834.
- (17) TISDALE, W. H., MELCHERS, L. E., and CLEMMER, H. J.
1927. STRAINS OF KERNEL SMUTS OF SORGHUM, SPHACELOTHECA SORGHI AND S. CRUENTA. *Jour. Agr. Research* 34: 825-838, illus.

PHYSIOLOGIC SPECIALIZATION IN PUCCINIA GRAMINIS SECALIS¹

By RALPH U. COTTER,² formerly Assistant Pathologist, and MOSES N. LEVINE, Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture³

INTRODUCTION

The literature dealing with the physiologic specialization of *Puccinia graminis secalis* Eriks. and Henn. and *P. dispersa secalis* Eriks. and Henn. is exceedingly meager. Levine and Stakman (4)⁴ reported in 1923 that *P. graminis secalis* comprised at least two, and probably three, distinct physiologic forms, identified by their parasitic effect on three commercial varieties of rye—Rosen, Swedish, and Prolific. Mains (6) in 1926 referred to two physiologic forms of *P. dispersa secalis*, which he distinguished by their parasitic effect on an inbred line of Abruzzes (Abruzzi) rye. This selfed line of rye was uniformly highly resistant to his physiologic form 1 and very susceptible to form 2. For obvious reasons the use of standard commercial varieties of cereals as differential hosts in the study of physiologic specialization of the grain rusts is highly desirable. But as rye naturally is cross-pollinated, the study of specialization in *P. graminis secalis* is more difficult than that of other varieties (races) of *P. graminis* for which pure lines of differential hosts may be obtained with comparative ease.

The particular object of the present investigation was to ascertain the number, geographic distribution, and parasitic nature of the physiologic forms of *Puccinia graminis* on rye. An attempt was made to obtain rust collections from as many localities as possible, to identify them, and to determine their parasitic behavior under varying conditions. Particular attention was paid to the rye-growing sections of the United States, the survey being especially concentrated in south-eastern Minnesota.

MATERIALS AND METHODS

The first serious endeavor to study intensively physiologic specialization in stem rust of rye was started in 1918. It was not until 1921, however, that the first three differential hosts, mentioned in the introduction, were found. Later, two additional differential varieties, Dakold and Colorless, were added. These five differentials were selected from a dozen or more commercial varieties and inbred lines of rye that had been inoculated repeatedly. In addition to the five differential varieties, each of which reacted differently to different physiologic forms, should be mentioned Giant Winter, obtained from J. F. Brandon, superintendent of the United States Dry-Land Field

¹ Received for publication Dec. 10, 1931; issued September, 1932. Cooperative investigation between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Agricultural Experiment Station of the University of Minnesota.

² Transferred to the Division of Barberry Eradication July 1, 1930.

³ The writers are indebted to E. C. Stakman, head of the Section of Plant Pathology of the Minnesota Agricultural Experiment Station, and pathologist in the U. S. Department of Agriculture, for suggestions during the course of the investigation. Thanks are due to the various colleagues who provided the writers with much of the inoculation material. The writers are especially appreciative of the cooperation of Albert C. Army, Division of Agronomy and Plant Genetics, Minnesota Agricultural Experiment Station, for supplying the seed used in this study except as otherwise specified in the text. The history and description of the differential varieties were supplied by H. K. Wilson, assistant professor of agronomy, University of Minnesota, and Manley Champlin, senior professor of field husbandry, University of Saskatchewan.

⁴ Reference is made by number (*italic*) to Literature Cited, p. 314.

Station at Akron, Colo. Giant Winter has proved extremely susceptible to all forms of *Puccinia graminis secalis* so far discovered and consequently can not be properly considered a differential host for these forms.

DESCRIPTION OF DIFFERENTIAL HOSTS

The five commercial varieties of rye chosen as differential hosts are all members of *Secale cereale* L. A brief description of each and of Giant Winter follows:

ROSEN, MINN. ACCESSION No. 82.—Rosen rye was developed at the Michigan Agricultural Experiment Station by selection from Petkus rye obtained from Russia through J. A. Rosen, agricultural expert of the Agrojoint Colonization Committee, in 1909. It was first distributed in 1912. It matures late, has large spikes filled with large dark kernels, is not so winter-hardy as Swedish No. 2, and is well adapted for growing in Michigan, Wisconsin, and southern Minnesota.

SWEDISH, MINN. No. 2.—A very hardy, good-yielding variety, which was produced at the Minnesota Agricultural Experiment Station by selection from a sample of Swedish rye obtained in 1895 from John Brogard, Henning, Minn. Seed was selected from the hardy plants that survived the winter. The seed was first distributed to farmers in 1907, and this is now the leading variety in Minnesota and the eastern part of South Dakota. The plants have small heads with small dark kernels, borne on rather tall, slender culms.

PROLIFIC, MINN. ACCESSION No. 89.—Prolific is a spring rye, the origin of which was a mass selection made at the University of Saskatchewan from an unknown sample of seed received from Germany. It was registered in 1921 under the name "Prolific, Sask. 302," and a quantity of hand-picked seed has been distributed each season since that time. According to Champlin (2), it matures very late but is a high yielder and is now grown extensively in the Province of Saskatchewan.

DAKOLD, MINN. ACCESSION No. 93.—Dakold rye was originated by the North Dakota Agricultural Experiment Station in 1902, when a few plants were found in a field where winter wheat had been sown but had been winterkilled. It was first distributed as "N. Dak. No. 959," but was later named Dakold. It resembles Swedish No. 2 in appearance and is very hardy under northwestern conditions. It is widely grown in North Dakota and has produced the highest average yield in a 4-year test at Saskatchewan.

COLORLESS, MINN. No. 104.—Colorless rye was produced at the Minnesota Agricultural Experiment Station by continued, pedigreed selection for the pale-color character from Swedish No. 2, resembling the latter in every other respect.

GIANT WINTER.—Giant Winter rye was introduced into the United States from France by the United States Department of Agriculture in 1901. The variety resembles Swedish No. 2 but appears to be of minor economic importance. It is grown to a certain extent in southeastern Wyoming and northeastern Colorado.

INOCULATION TECHNIC AND CULTURE METHODS

In all, 147 cultures of rye stem rust were studied. Specimens were obtained from 12 States in the United States, extending from Maine to Colorado and from Kansas to Wisconsin; from Ontario, Canada; and from France, Scotland, and Sweden. The hosts on which the rust was originally found comprised cultivated and volunteer rye, *Secale cereale*; the common barberry, *Berberis vulgaris* L.; and the following wild grasses: *Agropyron repens* (L.) Beauv., *A. smithii* Rydb., *A. tenerum* Vas., *Elymus* sp., *Hordeum jubatum* L., and *H. pusillum* Nutt. By means of inoculations, made for the past decade at University Farm, St. Paul, Minn., 104 of the cultures were separated into distinct physiologic forms. The inoculation technic was essentially that described by Stakman and Piemeisel (10). The identity of the forms was determined by a modification of the method used by Stakman and Levine (8) in their study of physiologic forms of *Puccinia graminis tritici* Eriks. and Henn. The various types of infection produced by *P. graminis secalis* on rye seedlings were used as a guide in the present study. (Fig. 1.) The host-reaction classes were

adapted with some modification from Levine (3). The modifications were necessary because of the heterozygous nature of the differential varieties of rye. This heterozygosity also necessitated, in the majority

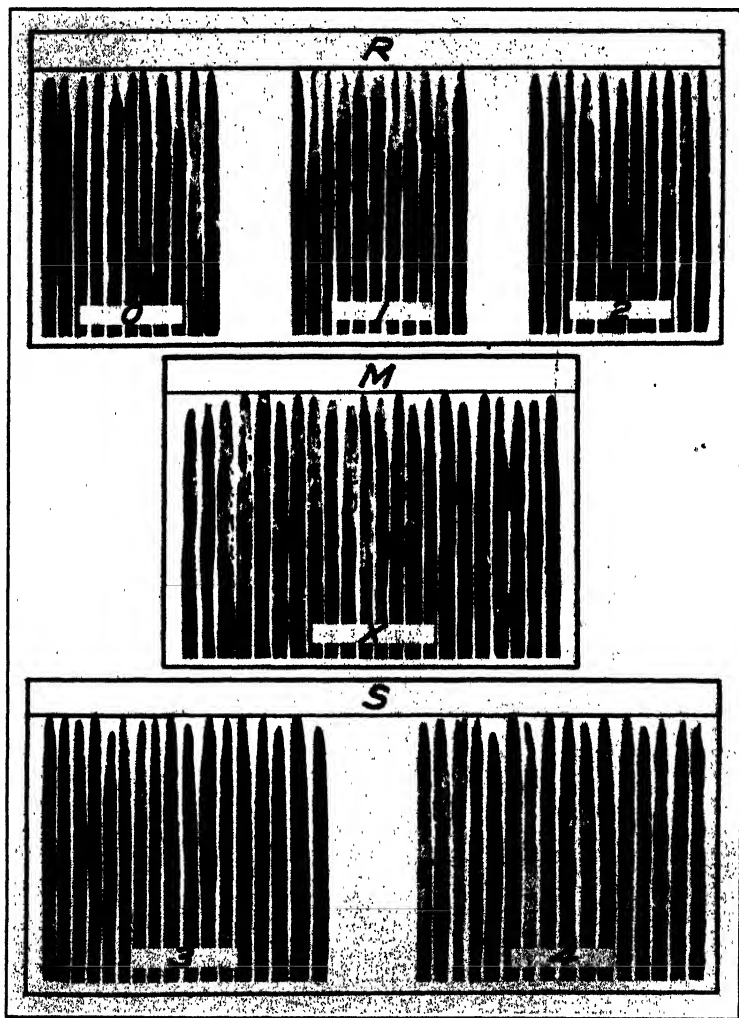


FIGURE 1.—Reaction classes of differential varieties of rye as indicated by infection types produced by *Puccinia graminis secalis*: Class R, resistant, embracing type 0 (no infection whatever or pronounced necrotic flecks), type 1 (minute uredia surrounded by solid necrotic areas), and type 2 (small pustules in green islands surrounded by necrotic halos); class M, mesothetic, consisting of type X (infection heterogeneous and ill defined); class S, susceptible, composed of type 3 (medium-size uredia with slight chlorosis but no necrosis), and type 4 (large confluent uredia, resulting in very heavy infection)

of cases, the testing of rather large numbers of individuals of each differential variety. On an average, more than 200 plants of each variety were infected before the identity of a rust culture was established, although there were some unavoidable exceptions.

CLASSIFICATION OF HOST REACTIONS

Three host-reaction classes—resistant, mesothetic, and susceptible—are recognized in the identification of physiologic forms of *Puccinia graminis secalis*. This classification is based on the different types of infection produced by the rust on seedlings and on the relative number of individuals in a given variety reacting in a definite manner. The following is a schematic description of the reaction classes and infection types:

CLASS R.—A variety is considered resistant when less than 25 per cent of the infected plants show normal development of rust, or conversely, when the rust-infection types produced on 75 per cent or more of the infected plants are, individually or severally, 0, 1, and 2, with their plus and minus fluctuations:

Type 0.—Plants virtually immune; no uredia are developed, but sharply defined necrotic flecks or necrotic lesions usually are present.

Type 1.—Plants extremely resistant; infection very light; uredia minute and scattered, surrounded by clearly defined, continuous necrotic areas.

Type 2.—Plants moderately resistant; infection light; uredia isolated and small to medium in size; hypersensitive areas in the form of necrotic halos or circles; pustules usually in green, though slightly chlorotic, islands.

CLASS M.—The host reaction is considered mesothetic when more than 25 and less than 75 per cent of the infected plants have rust pustules of types 3 and 4, or when the infection on all or most of the plants is heterogeneous as indicated by infection type X with its accompanying plus and minus fluctuations:

Type X.—Infection heterogeneous; uredia very variable, apparently including all types and degrees of infection on the same blade; no mechanical separation seems possible, since on reinoculation spores from small uredia may produce large ones, and vice versa.

CLASS S.—When 75 per cent or more of the infected plants of a given variety are moderately to heavily rusted, i. e., when the infection on them is of either type 3 or 4, or both, including their plus and minus variations, the variety is designated susceptible:

Type 3.—Plants moderately susceptible; infection medium; uredia mid sized with slight tendency to coalesce; true hypersensitiveness absent but light chlorotic areas usually present, especially under unfavorable cultural conditions.

Type 4.—Plants completely susceptible; infection normal and heavy; uredia large and generally confluent; hypersensitiveness normally absent, but chlorosis may be present when cultural conditions are not favorable.

Plus and minus signs indicate a slightly greater or smaller amount of rust than the nearest figure representing the infection type. Necrotic lesions are designated by a dot (.), necrotic flecks by a semi-colon (;), and necrotic islands by a colon (:). The sign of equality denotes double minus.

The determination of the rust reaction of a given variety to *Puccinia graminis secalis* is rather simple when a great many plants are tested. The individual plants are examined first for infection types; then they are grouped according to the reaction classes, and the number in each class is recorded. The relative susceptibility of the variety is determined by the quotient obtained from dividing the number of susceptible plants by the total number of infected plants. Thus, for example, Colorless was considered highly susceptible to the Litchfield (Minn.) culture (form 11), because 461, or 91.47 per cent, of the 505 plants that became infected had rust pustules of types 3 and 4; of the remaining infected plants, 43, or 8.51 per cent, were placed in the resistant class, and 1 was classified as mesothetic. Dakold, on the other hand, was considered resistant to this culture, because only 25, or 8.93 per cent, of the 280 infected plants were really susceptible. Similarly, the Swedish variety was classified as mesothetic to the Litchfield culture because 32.08 per cent of the infected plants, i. e., more than 25 but less than 75 per cent, were heavily infected.

However, when the number of plants was unavoidably small, the susceptibility quotient ceased to be effectively operative. In such cases a numerical equivalent, as presented in Table 1, was assigned to each infected plant according to the degree and type of infection produced on it. From these the relative susceptibility of the variety was determined by calculating the average percentage equivalent. For instance, in the case of Rosen, inoculated with the culture from Haddington, Scotland (form 11), 19 plants became infected; 3 of these were marked type 3, 3 were 4-, and the remaining 13 were recorded as 4+. The total numerical equivalent for the 19 plants, according to Table 1, was $3 \times 50 + 3 \times 82 + 13 \times 94 = 1,618$, with an average for the variety of $1,618 \div 19$, or 85.16 per cent.

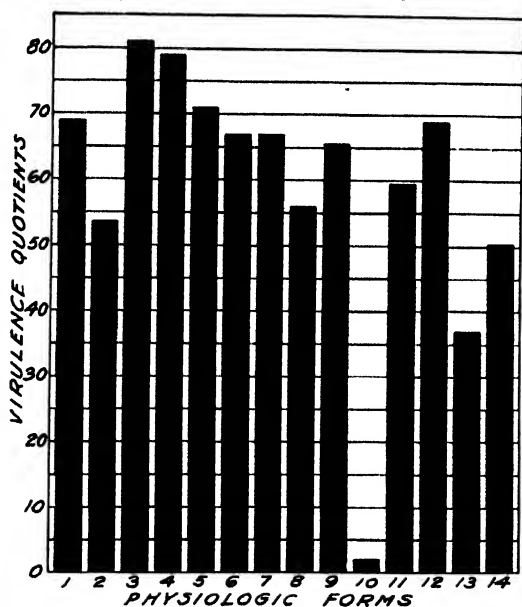


FIGURE 2.—Successive series of the physiologic forms of *Puccinia graminis secalis* indicating their respective virulence quotients

TABLE 1.—Graduated scale showing numerical equivalents for host reactions, corresponding to symbols representing types and degrees of infection,^a used as a means to determine the relative susceptibility of differential varieties

Immune to moderately resistant				Mesothetic to extremely susceptible			
Infection index	Numerical equivalent	Infection index	Numerical equivalent	Infection index	Numerical equivalent	Infection index	Numerical equivalent
0	0	x.	8	x=	28	x+	65
0;	1/2	x;	10	x-	32	x++	70
0:	1	2=	12	x	36	4=	76
1=	2	2-	15	3=	40	4-	82
1-	3	2	18	3-	45	4	88
1+	4	2+	21	3	50	4+	94
1++	5	2++	24	3+	55	4++	100
1+++	6			3++	60		

^a For explanation of symbols see p. 300.

[illegible]

TABLE 3.—*Relative susceptibility of differential varieties of Secale cereale and comparative virulence of physiologic forms of Puccinia graminis secalis*

Physiologic form No.	Relative susceptibility of—										Virulence quotient
	Rosen		Swedish		Prolific		Dakold		Colorless		
	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	
1	0.43-24.87	17.91	74.54-94.00	86.80	82.47-93.00	95.45					68.67
2	78.57-94.87	88.06	3.40-21.43	15.79	37.50-41.46	40.00					53.53
3	78.44-94.00	88.47	77.00-88.00	83.42	76.14-88.00	81.36	56.34-71.43	60.92	54.50-91.67	83.34	59.18
4	84.71-95.05	93.32	79.02-88.00	81.78	57.04-72.94	62.63	60.05-65.34	61.25	65.65-84.00	98.88	
5	82.30-85.29	84.29	2.00-19.00	6.96	83.33-88.00	85.67					70.80
6	82.00-94.00	84.21	98.00-96.00	93.55	.87-22.75	17.39					67.12
7	77.04-90.48	89.29	43.96-71.66	62.72	44.57-71.30	60.35	26.17-53.63	38.01	77.24-90.63	88.76	66.90
8	54.98-74.25	67.75	30.83-51.70	41.41	57.28-68.35	62.13	10.67-19.13	13.58	75.31-98.89	79.43	56.24
9	77.78-91.19	87.32	40.66-61.80	52.39	76.89-84.86	81.21	11.18-22.44	19.62	75.60-94.04	86.72	65.55
10	1.00-2.00	1.85	1.00-3.00	2.22	1.00-3.00	2.50					2.13
11	79.37-92.11	87.35	32.04-61.89	46.70	37.50-71.65	58.68	5.56-23.32	17.35	76.72-93.62	86.60	59.53
12	79.61-92.67	86.06	53.63-70.81	63.54	76.07-87.61	81.20	26.82-67.39	36.73	81.55-98.00	87.79	69.18
13	25.59-56.49	36.79	15.37-24.13	20.80	31.25-71.96	47.40	2.54-13.24	4.68	49.75-71.27	63.84	37.22
14	83.33-86.13	85.75	39.00-40.11	39.87	43.79-60.28	53.85	10.19-13.88	10.82	65.87-70.49	65.29	50.37
Weighted average	85.88		54.85		63.96		28.29		86.55		63.57

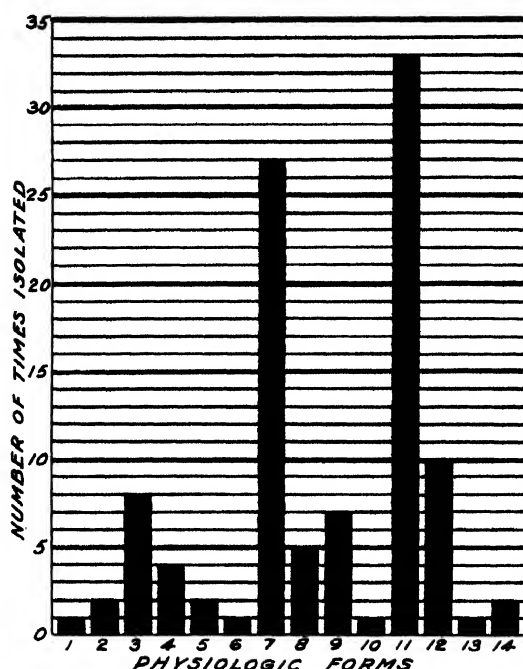


FIGURE 3.—Frequency distribution of physiologic forms of *Puccinia graminis secalis* identified during the period 1921–1931

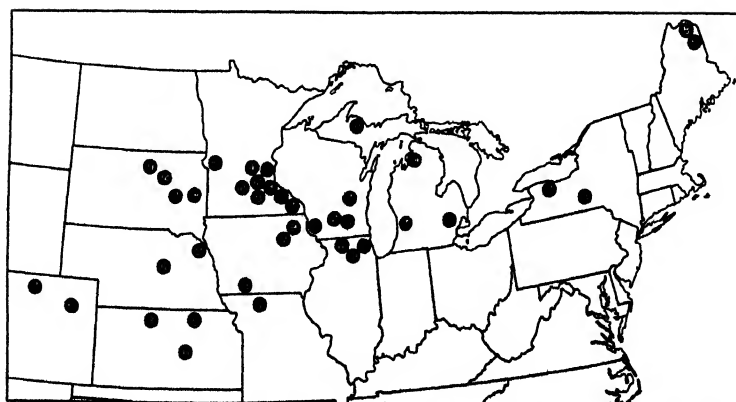


FIGURE 4.—Distribution of physiologic forms (numerals in circles) of *Puccinia graminis secalis* in the United States as represented by individual collections made and identified during the period 1921–1931

Data on the prevalence and distribution of the physiologic forms are presented in Figures 3 and 4 and in the following list:

Places where the different physiologic forms of Puccinia graminis secalis were collected, 1921-1931, and the number of times each was isolated

Times isolated		Times isolated	
Form 1.....	1	Form 9.....	7
1921—St. Paul, Minn.		1924—Wanamingo, Minn.	
Form 2.....	2	1926—Marengo, Ill.	
1922—Madison, Wis.		Ponca, Nebr.	
Massy, France.		1927—Decorah, Iowa.	
Form 3.....	8	1931—Cherrygrove, Minn.	
1921—Gurnee, Ill.		Kenyon, Minn.	
1922—Caledonia, Ontario.		Union Lake, Minn.	
Gurnee, Ill.		Form 10.....	1
Ithaca, N. Y.		1923—Akron, Colo.	
Marshall, Wis.		Form 11.....	33
Northfield, Minn.		1922—Haddington, Scotland.	
Red Wing, Minn.		Rochester, N. Y.	
1931—Rosemount, Minn.		St. Paul, Minn.	
Form 4.....	4	1924—Redding, Iowa.	
1922—Briançon, France.		1925—Belleville, Kans.	
Minneapolis, Minn.		Litchfield, Minn.	
1927—Northfield, Minn.		1926—East Lansing, Mich.	
1930—Minneapolis, Minn.		Minneapolis, Minn.	
Form 5.....	2	Petoskey, Mich.	
1922—Presque Isle, Me.		Salina, Kans.	
1923—Commerce, Mich.		Wells, Minn.	
Form 6.....	1	Woodstock, Ill.	
1922—Stockholm, Sweden.		1927—Belleville, Kans.	
Form 7.....	27	Decorah, Iowa.	
1925—Gilman City, Mo.		Rochester, Minn.	
Red Wing, Minn.		Traverse City, Mich.	
1926—Bancroft, S. Dak.		1928—New Brighton, Minn.	
Charlevoix, Mich.		Zumbra Heights, Minn.	
Chatham, Mich.		1929—Chatfield, Minn.	
Fort Collins, Colo.		Minnetrista, Minn.	
Lake City, Minn.		Orono, Minn.	
Sauk Center, Minn.		Rochester, Minn.	
1927—Belleville, Kans.		Rosetown, Minn.	
Bloomington, Wis.		St. Paul, Minn.	
Cherrygrove, Minn.		1930—Minneapolis, Minn. (twice)	
Decorah, Iowa.		1931—Belleville, Kans.	
Ganges, Mich.		Faribault, Minn.	
Lebanon, Kans.		Ishpeming, Mich.	
Lesueur Center, Minn.		Loyalton, S. Dak. (twice).	
Lewiston, Minn.		Mankato, Minn.	
Plainview, Minn.		Marquette, Mich.	
Red Wing, Minn.		Form 12.....	10
Salina, Kans.		1922—Minneapolis, Minn.	
Saratoga, Minn.		1925—Redfield, S. Dak.	
1929—Chatfield, Minn.		1928—Minneapolis, Minn.	
Rochester, Minn.		1931—Haycreek, Minn.	
1930—Lydia, Minn.		Invergrove, Minn.	
1931—Anoka, Minn.		Northfield, Minn. (twice).	
Blooming Prairie, Minn.		Ord, Nebr.	
Faribault, Minn.		Potosi, Wis.	
Fond du Lac, Wis.		Westfield, Minn.	
Form 8.....	5	Form 13.....	1
1922—Presque Isle, Me.		1926—Morris, Minn.	
1924—Belleville, Kans.		Form 14.....	2
Goodhue, Minn.		1926—Central Lake, Mich.	
1927—Belleville, Kans.		1927—Brookings, S. Dak.	
1931—Invergrove, Minn.			

The number of forms identified in each year was as follows: In 1921, 2; in 1922, 8; in 1923, 2; in 1924, 3; in 1925, 3; in 1926, 5; in 1927, 6; in 1928, 2; in 1929, 2; in 1930, 3; in 1931, 6; total, 14.

It is noteworthy that each of all but two of the collections studied consisted of a single physiologic form. The exceptions were telial collections on *Agropyron repens*, one from Decorah, Iowa, and the other from Chatfield, Minn. *Berberis vulgaris* was inoculated with sporidia of these collections, and the resulting aecia yielded three distinct forms from the Decorah collection (forms 7, 9, and 11) and two forms from the Chatfield collection (forms 7 and 11).

PHYSIOLOGIC SPECIALIZATION

When the type and degree of infection and the relative susceptibility of each differential variety have been determined, the physiologic forms are identified with the aid of a key similar to those used for the identification of physiologic forms of *Puccinia graminis tritici* and *P. graminis avenae* (8, 1). The key is here included. Only the reaction class of each variety is considered. For example, if Rosen is resistant and Swedish susceptible, the key would indicate that the culture producing this reaction is *P. graminis secalis* form 1. On the other hand, if Rosen is susceptible, Swedish and Prolific mesothetic, Dakold resistant, and Colorless susceptible, the rye stem rust causing these reactions would be identified as form 11. The key does not indicate the reaction of the complete set of differentials in every instance. It is therefore necessary to check the tentative identification with the known action of the corresponding forms as indicated in Table 3. If the relative susceptibility of each of the differential hosts approximates that designated for the physiologic form in question, the identification is considered complete; if it does not, then either the form has not yet been described or the culture consists of a mixture of forms, which must be separated before correct identification can be made.

IDENTIFICATION OF PHYSIOLOGIC FORMS

Analytical key for the identification of physiologic forms of *Puccinia graminis secalis* on the basis of their parasitic behavior on five differential varieties within the species *Secale cereale*.

	Form No.
Rosen resistant:	
Swedish resistant.....	10
Swedish susceptible.....	1
Rosen mesothetic:	
Swedish resistant.....	13
Swedish mesothetic.....	8
Rosen susceptible:	
Swedish resistant—	
Prolific mesothetic.....	2
Prolific susceptible.....	5
Swedish mesothetic —	
Prolific mesothetic —	
Dakold resistant —	
Colorless mesothetic.....	14
Colorless susceptible.....	11
Dakold mesothetic.....	7
Prolific susceptible—	
Dakold resistant.....	9
Dakold mesothetic.....	12
Swedish susceptible—	
Prolific resistant.....	6
Prolific mesothetic.....	4
Prolific susceptible.....	3

PATHOGENICITY

It will be seen from Tables 2 and 3 that some of the physiologic forms of *Puccinia graminis secalis* are very virulent, whereas others are extremely weak on the differential varieties of rye. The behavior of the first 3 of the 14 identified forms on Rosen, Swedish, and Prolific has been briefly described by Levine and Stakman (4) as follows:

One form [form 3] is quite virulent, possessing the capability of attacking heavily all of the three varieties; another form [form 2] attacks Rosen very heavily, Prolific only moderately and Swedish very weakly; while still another form [form 1] produces normal infection on Swedish and Prolific, but only weak infection on Rosen.

Judging from its virulence quotient (Table 3 and fig. 2), it would seem that form 3 is the most virulent of all known forms, but only slightly more so than form 4. Next in order of virulence is form 5. It must be remembered, however, that the reaction of Dakold and Colorless to this form is unknown; consequently its exact virulence quotient is not fully established. Form 12, with a virulence quotient of 69.18, follows very closely behind form 5, whose quotient is 70.80 on the basis of the three varieties tested. Each of these quotients is considerably lower than that of form 3, whose quotient is 80.55, or that of form 4, whose quotient is 79.18. Unlike form 5, forms 3, 4, and 12 have been tested on all of the differentials. The weakest in virulence, as far as has been determined, is form 10, whose quotient is only 2.13, the next being form 13 with a quotient of 37.22. However, it must be borne in mind that form 10, like form 5, has been tested on only three differential hosts.

PREVALENCE AND DISTRIBUTION

More than half of all collections identified consisted of only forms 7 and 11. These two forms in the main closely resemble each other in their pathogenicity on the five differentials. The only outstanding difference is furnished by Dakold, a variety mesothetic to Form 7 and resistant to form 11. The difference in virulence quotient of the two forms is slightly more than 7 points. Forms 7 and 11 resemble each other not only in frequency distribution, 27 and 33 isolations respectively (see p. 309, Table 4, and fig. 3), but also in extent of distribution geographically. Both forms were for the most part found in only three States—Kansas, Michigan, and Minnesota (Table 4); form 7 was collected in these States twenty-one times, and form 11, twenty-six times. In addition, form 7 was found also in Colorado, Iowa, Missouri, South Dakota, and Wisconsin; while form 11 was collected also in Illinois, Iowa, New York, South Dakota, and Scotland. Form 7 was isolated twice in 1925, six times in 1926, twelve times in 1927, twice in 1929, once in 1930, and four times in 1931, whereas form 11 appeared three times in 1922, once in 1924, twice in 1925, six times in 1926, four times in 1927, twice in 1928, six times in 1929, twice in 1930, and seven times in 1931.

TABLE 4.—Prevalence and distribution of physiologic forms of *Puccinia graminis secalis* identified during the period 1921–1931

Distribution	Number of times indicated form was found														Total number of—	
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	Cultures	Forms
United States:																
Colorado.....							1			1					2	2
Illinois.....			2						1		1				4	3
Iowa.....							1		1		2				4	3
Kansas.....							3	2			4				9	3
Maine.....					1			1							2	2
Michigan.....					1		3				5			1	10	4
Minnesota.....	1		3	3			15	2	4		17	7	1		53	9
Missouri.....							1					1			1	1
Nebraska.....									1						2	2
New York.....			1				1				1	1			2	2
South Dakota.....							1				2	1		1	5	4
Wisconsin.....		1	1				2								5	4
Foreign countries:																
Canada.....			1												1	1
France.....		1		1											2	2
Scotland.....											1				1	1
Sweden.....						1									1	1
Total.....	1	2	8	4	2	1	27	5	7	1	33	10	1	2	104	14

The only other forms that were fairly common, besides those just discussed, were forms 3 and 12. Form 3 was isolated only during three years of the investigation, once in 1921, six times in 1922, and once in 1931, but it occurred in New York, also through Ontario to Minnesota, in an almost straight line. Form 12 was found once each in 1922, 1925, and 1928, but was isolated seven times in 1931. As is true of the physiologic forms of stem rusts of wheat and of oats (3, 5, 9), the same forms of *Puccinia graminis secalis* did not recur with any regularity year after year, nor were they uniformly distributed in any definite area.

All the forms except form 6 have been found in the United States. Form 6 came from Stockholm, Sweden. However, since this study by no means constitutes a complete survey of the status of rye stem rust, it is unlikely that all of the forms that may have been present were collected.

DISCUSSION AND CONCLUSIONS

Notwithstanding the heterozygous nature of commercial varieties of rye, it was possible to establish the existence of at least 14 physiologic forms of *Puccinia graminis secalis* by the use of certain standard varieties of *Secale cereale*. In many cases great numbers of plants must be tested to be certain of the results. However, when the results of inoculation are clear-cut and uniform, smaller numbers may suffice. In the identification of form 7, for example, where three of the five differential varieties are mesothetic or intermediate in reaction, not less than 200 seedlings of each variety were found to be necessary. In the case of form 10, on the other hand, apparently reliable results were obtained with a tenth of the required number of plants tested. The infection types in the last instance were very definite and absolutely uniform. The maximum infection on any one plant was (1-), the minimum (0;). It seemed reasonable to accept these results as conclusive and as a basis for considering the

existence of form 10 as highly probable. That this form is a component part of *P. graminis secalis* is evidenced by the fact that the original culture came from heavily infected Giant Winter rye. At any rate, the majority of the forms described in this paper were identified after a considerable number of individuals of the various differential hosts had been tested. Forms 7 and 11 were found most widely distributed and of most frequent occurrence, representing about 26 and 32 per cent, respectively, of the total number of cultures identified. Form 12 was third, with a 10 per cent ratio in the frequency distribution, and form 3 was fourth, with approximately 8 per cent of the total number identified. The remaining 11 forms gradually tapered down from seven isolations to only a single one.

Seventy-four of the collections were identified directly from the original material, for they were either in the uredial (46 cultures) or aecial (28 cultures) stages. The remaining 30 cultures were of telial origin and had to pass through the aecial stage on barberry (*Berberis vulgaris* L., *B. oblonga* Schn., or *B. notabilis* Schn.) before their behavior on the differentials could be ascertained. It was from two telial collections on quack grass that three different forms (7, 9, and 11), in one case, and two forms (7 and 11), in another case, were isolated on passing through the aecial stage on the common barberry. Inasmuch as it is not known whether the original material in either case consisted of a single physiologic form, it would be hazardous to assume that a genetic change had occurred. There is evidence, however, of hybridization in the aecial stage, resulting from crossing *Puccinia graminis secalis* with *P. graminis tritici*.

Changes in environmental conditions affect perceptibly the degree of infection and virulence quotients of forms of rye stem rust but not their physiologic specialization. Pathogenically these forms appear to remain genetically constant under varying conditions of temperature and luminescence. L. W. Melander⁵ observed yellow pustules in a brown culture of rye stem rust, supplied to him by the writers and grown by him under constant light (162 foot-candles) at 20° C. The yellow pustules also were present at the same time in the original culture grown by the writers under ordinary greenhouse conditions. This would tend to show the presence of color mutation in *Puccinia graminis secalis*, similar to that described by Newton and Johnson (7) for *P. graminis tritici*. Extensive tests proved the two varicolored cultures to be pathogenically identical, namely, *P. graminis secalis* form 7.

Many varieties of barley and all the differential varieties of wheat, as well as the oat differentials, were subjected to a thorough test with the *secalis* forms. The oat varieties were extremely resistant throughout. All the differential varieties of wheat reacted in like manner, except Little Club. This variety reacted heterogeneously to a few collections of *Puccinia graminis secalis* when first inoculated. In all these tests the presence of forms of *P. graminis tritici* was detected. In one instance, however, the presence of the wheat stem rust was not discovered until after many tests had been made. Its occurrence may therefore have resulted from a subsequent accidental contamination, and the moderate development of rust on Little Club evidently was entirely due to the rye rust. Unfortunately, this rust strain was

⁵ Associate Pathologist, Division of Barberry Eradication, Bureau of Plant Industry.

destroyed before monosporous cultures could be obtained for further intensive analysis. However, reasonably pure cultures of forms 7 and 11 of *Puccinia graminis secalis* recently have been identified that infected Little Club wheat in an indeterminate (mesothetic) manner, although all other wheat differentials definitely proved to be extremely resistant to these forms.

Between 30 and 40 varieties of barley belonging to the species *Hordeum vulgare* L., *H. intermedium* Kcke., *H. distichon* L., and *H. deficiens* Steud., reacted variously to the different tested forms of *Puccinia graminis secalis*. A detailed study of the reaction of barley varieties to the different cereal rusts is at present being completed.

SUMMARY

During the period from 1921 to 1931, inclusive, close to 150 collections of *Puccinia graminis secalis* were studied to determine the physiologic specialization within this rust variety.

Fourteen physiologic forms have been distinguished, and the characteristic parasitic behavior of each has been ascertained on several differential varieties of *Secale cereale*.

Because rye is cross-pollinated, the identification technic is more involved than in the case of stem rust of either wheat or oats, but apparently is not less certain.

Some of the physiologic forms identified occurred frequently and were widely distributed, others occurred rarely and in restricted areas.

Form 11 was isolated from the largest number of localities and was an important factor in the rye stem-rust epidemics of 1926, 1927, 1929, and 1931, occurring with approximately even frequency in all these years except 1927.

Although form 7 was collected almost as often as form 11, it appeared to be scattered over a greater geographic area and was isolated at different times for six, instead of nine, years of the present investigation. It was especially common in 1927.

Forms 1, 6, 10, and 13 were collected only once each; the remaining eight forms were isolated from two to ten times each.

The frequency of the occurrence of the different physiologic forms was not always coextensive with their distribution, nor was the prevalence of a given physiologic form paralleled by its virulence on the differential varieties.

The pathogenicity of the physiologic forms of *Puccinia graminis secalis* was but slightly and only temporarily affected by external conditions, such as temperature and light.

There seems to be strong circumstantial evidence of the occurrence of color mutations in the rye stem rust, but none so far of mutations in parasitic behavior.

The possibility of the origin of rye stem-rust forms through hybridization in the aecial stage is supported by the evidence of the production of new forms of wheat stem rust by crossing *Puccinia graminis secalis* with *P. graminis tritici*.

LITERATURE CITED

(1) BAILEY, D. L.

1925. PHYSIOLOGIC SPECIALIZATION IN 'PUCCINIA GRAMINIS AVERNAE' ERIKSS. AND HENN. Minn. Agr. Expt. Sta. Tech. Bul. 35, 33 p., illus.

- (2) CHAMPLIN, M.
1927. RYE PRODUCTION IN SASKATCHEWAN. Saskatchewan Univ. Col. Agr. Ext. Bul. 35, 20 p., illus.
- (3) LEVINE, M. N.
1928. BIOMETRICAL STUDIES ON THE VARIATION OF PHYSIOLOGIC FORMS OF PUCCINIA GRAMINIS TRITICI AND THE EFFECTS OF ECOLOGICAL FACTORS ON THE SUSCEPTIBILITY OF WHEAT VARIETIES. Phytopathology 18: 7-123, illus.
- (4) ——— and STAKMAN, E. C.
1923. BIOLOGIC SPECIALIZATION OF PUCCINIA GRAMINIS SECALIS. (Abstract) Phytopathology 13: 35.
- (5) ——— STAKMAN, E. C., and STANTON, T. R.
1929. FIELD STUDIES OF THE RUST RESISTANCE OF OAT VARIETIES. U. S. Dept. Agr. Tech. Bul. 143, 36 p., illus.
- (6) MAINS, E. B.
1926. STUDIES IN RUST RESISTANCE. Jour. Heredity 17: 313-325, illus.
- (7) NEWTON, M., and JOHNSON, T.
1927. COLOR MUTATIONS IN PUCCINIA GRAMINIS TRITICI (PERS.) ERIKSS. AND HENN. Phytopathology 17: 711-725, illus.
- (8) STAKMAN, E. C., and LEVINE, M. N.
1922. THE DETERMINATION OF BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON TRITICUM SPP. Minn. Agr. Expt. Sta. Tech. Bul. 8, 10 p., illus.
- (9) ——— LEVINE, M. N., and WALLACE, J. M.
1929. THE VALUE OF PHYSIOLOGIC-FORM SURVEYS IN THE STUDY OF THE EPIDEMIOLOGY OF BLACK STEM RUST. Phytopathology 19: 951-959, illus.
- (10) ——— and PIEMEISEL, F. J.
1917. BIOLOGIC FORMS OF PUCCINIA GRAMINIS ON CEREALS AND GRASSES. Jour. Agr. Research 10: 429-496, illus.

LEAD ARSENATE POISONING IN CHICKENS¹

By E. F. THOMAS, *Assistant Veterinarian*, and A. L. SHEALY, *Head, Department of Animal Husbandry and Dairying, Florida Agricultural Experiment Station*

INTRODUCTION

During the spring of 1929 the veterinarians of the Florida experiment station staff were called upon to investigate numerous complaints from poultry owners who lived in areas of Florida in which lead arsenate spray was being used in combating the Mediterranean fruit fly. Since it was impossible to obtain information on the toxicity of lead arsenate to chickens, and there were few indications of poisoning in the greater number of cases, it was necessary to conduct tests with lead arsenate and lead arsenate spray solution to determine their effect on chickens.

The spray formula used by the United States Department of Agriculture in cooperation with the Florida State plant board in the campaign against the Mediterranean fruit fly was as follows: Lead arsenate, 8 pounds; brown sugar, 50 pounds; syrup, 10 gallons; and water sufficient to make 200 gallons. In all tests with poultry the solution was prepared according to this formula, and made up in 1-gallon quantities.

EXPERIMENTAL DATA

The tests to determine the effect of powdered lead arsenate on chickens were conducted first. Each chicken was assigned a number, weighed, and fed a definite quantity of lead arsenate powder. Observations were made several times daily after the feeding. In all cases the lead arsenate was given in capsules except to the birds receiving 1-ounce and 2-ounce doses, where it was necessary to add the powder to water and administer as a solution. Table 1 shows the quantity given each bird and the results obtained. Birds used as checks in adjacent pens were all alive and healthy at the termination of these tests.

To determine the effects of allowing chickens to have access to lead arsenate spray solution continually both as drink and food, the following tests were conducted.

Six frying-size chickens of mixed breeding, two of which weighed 2 pounds each and four of which weighed 3 pounds each, were placed in pen No. 1, July 1, 1929. These birds were allowed nothing to drink but lead arsenate spray solution. They were fed mash and scratch grain as usual. A gallon of the solution was made up daily and placed before them in an inverted type of fountain. This type of drinking fountain assured the consumption of the stronger solution, since lead arsenate has a tendency to settle to the bottom on standing. The six birds consumed approximately 1 quart daily. The test lasted for 60 days, ending September 1, 1929. At the end of the test period all the birds showed a normal gain in weight and were apparently normal in every other respect.

¹ Received for publication Jan. 19, 1932; issued September, 1932.

TABLE 1.—Results of feeding various quantities of powdered lead arsenate to chickens

Bird No.	Weight	Date fed lead arsenate	Quantity of lead arsenate fed	Effects	Bird No.	Weight	Date fed lead arsenate	Quantity of lead arsenate fed	Effects
	<i>Ounces</i>		<i>Grains</i>			<i>Ounces</i>		<i>Grains</i>	
311.....	52	June 21	20	Died June 29.	383.....	16	June 21	80	Died June 25.
377.....	20			Lived.	74.....	24			Died June 29.
381.....	20			Do.	380.....	28			Lived.
340.....	40			Do.	348.....	36			Do.
392.....	48		40	Do.	53.....	48		160	Do.
342.....	56			Do.	62.....	76			Died July 18.
384.....	18			Lived.	375.....	52			Died July 1.
343.....	24			Died June 27.	376.....	72			Died July 2.
329.....	28		60	Died July 1.	38.....	100	June 22	240	Died June 29.
364.....	44			Lived.	310.....	16			Died June 24.
363.....	48			Do.	361.....	20			Died June 25.
382.....	56			Do.	368.....	24			Died June 24.
338.....	24			Died July 1.				<i>Ounces</i>	
386.....	20			Died July 4.	302.....	32			Died July 2.
369.....	20			Died July 6.	344.....	40			Died June 27.
					359.....	40			Lived.
					136.....	112		2	Died June 27.

Six apparently healthy Buff Leghorn hens were placed in pen No. 2 on July 3, 1929. These birds were allowed nothing to eat except scratch grain that had been soaked in lead arsenate spray solution. They were given a quart of fresh soaked scratch feed daily, but the quantity consumed was not determined since a great deal was wasted by their scratching and pecking it out onto the ground. This test extended over a 60-day period and ended September 3. All six birds were apparently in good health at the end of the 60 days. Birds used as checks in adjacent pens were all alive and apparently in good health at the termination of the tests.

DISCUSSION

In pen No. 1 each bird consumed approximately 13 grains of lead arsenate daily, a total consumption of 780 grains over a 60-day period, and showed no symptoms of poisoning.

Birds in pen No. 2, although given only scratch feed, apparently suffered no ill effects from consuming a small amount of lead arsenate daily for 60 days.

Of 31 birds fed powdered lead arsenate, 18 died from lead arsenate poisoning; of 6 fed 20 grains, 1 died; of 6 fed 40 grains, 2 died; of 3 fed 60 grains, 3 died; of 6 fed 80 grains, 3 died; of 3 fed 160 grains, 3 died; of 3 fed 240 grains, 3 died; of 3 fed 1 ounce, 2 died; and the only bird fed 2 ounces died. (Table 1.) Death was produced in 2 to 27 days, the average being 9 days. The cause of death in each case was determined by post-mortem examination.

The symptoms of the birds poisoned by lead arsenate were drowsiness, thirst, loss of appetite, diarrhea, and, in the last stages, cyanotic combs and wattles. A few of the birds passed several ascarids during the first 48 hours after they were fed the lead arsenate. On autopsy no differences were noted in the severity of the poisoning in those birds that passed ascarids and those that did not.

On post-mortem examination, birds fed the larger amounts of lead arsenate showed some of the material still in the crop and gizzard,

and in such birds necrotic areas were found in the crop lining. Other post-mortem lesions were dark congested liver, congested and hemorrhagic intestinal mucosa with blood-stained contents and a large amount of mucus. The kidneys were pale in color.

All birds used in these lead arsenate tests that were apparently healthy at the end of the experiment were used in other kinds of tests, and post-mortem examinations made on them during the next year did not reveal any lesions that were associated with lead arsenate poisoning.

Since it would seem reasonable to expect decreased egg production and loss of weight in hens on restricted diets such as were fed to those in pens No. 1 and 2, it was not deemed necessary to consider these factors in these tests.

In the Mediterranean fruit-fly campaign approximately 7 gallons of lead arsenate spray solution was used on an acre of citrus. The results of the experiments here reported indicate that 168 hens could consume this amount continually for 60 days and suffer no ill effects. It would seem, therefore, that it is impossible for chickens to consume enough solution or sprayed foliage in a sprayed grove to suffer from the effects of lead arsenate poisoning.

CONCLUSIONS

Lead arsenate will produce death in chickens when fed in large quantities.

There appeared to be no definite correlation between the weights of the birds and a lethal dose of lead arsenate.

Lead arsenate spray solution of the strength used in these tests is apparently not harmful to chickens when consumed with feed and water continually for 60 days. A bird may consume as much as 13 grains per day for 60 days without suffering any ill effects.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 45

WASHINGTON, D. C., SEPTEMBER 15, 1932

No. 6

ROSE ANTHRACNOSE CAUSED BY SPHACELOMA¹

By ANNA E. JENKINS²

Associate Pathologist, Division of Mycology and Disease Survey, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The results of an investigation of the widespread but comparatively little-known anthracnose of the rose (*Rosa*) are reported herein, and the literature on the subject is assembled for the first time, so far as the writer knows. The investigation, begun in 1925, embodies a study of the disease mainly as found in the test gardens of the American Rose Society, at Cornell University, Ithaca, N. Y., and in the Van Fleet rose collection at the United States Plant Field Station at Glenn Dale, Md. During August and September, 1930, observations were made on a number of rose plantings in England. There has also been available for study diseased material from rose plantings in Japan, New South Wales, Portugal, and elsewhere, together with that intercepted by Federal plant quarantine inspectors from European importations of rose³ (30, 44)⁴ and that found in mycological and phanerogamic herbaria in the United States, England, and Scotland. The intercepted material includes part of a rose bouquet brought to the United States by a passenger on the German dirigible *Graf Zeppelin* on its first trip to this country, in October, 1928 (30, 31, 34, 43). (Fig. 1, A.)

ROSE SPECIES AND VARIETIES AFFECTED

The disease discussed herein affects stems, leaves, and inflorescence of the rose, but in practically all cases it has been reported only on leaves of this plant. Discussing the disease on rose leaves in New South Wales in 1903, Cobb (10, p. 1064) stated that "rank growing roses, producing large and succulent leaves, are the worst sufferers" and that "some varieties * * * particularly * * * small-leaved and climbing sorts appear almost never to suffer from the disease."

Among the numerous species affected are: *Rosa acicularis engelmanni* Engel., *R. blanda* L., *R. canina* L., *R. centifolia* L., *R. chinensis*

¹Received for publication Jan. 20, 1932; issued October, 1932. Many of the data herein presented were included in a thesis submitted to the faculty of the Graduate School of Cornell University, June, 1927, in partial fulfillment of the requirements for the degree of doctor of philosophy.

²Thanks are due Prof. L. M. Massey, who first called the writer's attention to the disease under discussion, and Prof. H. M. Fitzpatrick, both of the Department of Plant Pathology, Cornell University, and to Prof. A. J. Eames, of the Department of Botany of the same university, under whose direction the present investigation was carried out. The writer appreciates also the criticism of the manuscript by E. J. Butler and E. W. Mason, both of the Imperial Mycological Institute, Kew, England. Acknowledgment is likewise made of the assistance given by other mycologists, including N. L. Alcock, W. Buddin, R. C. Harris, M. H. Moore, W. C. Moore, J. Ramsbottom, A. Smith, T. A. Sprague, and E. M. Wakefield, of Great Britain; Mathilde Bensaude, of Portugal; J. G. Churchward, T. H. Harrison, and W. L. Waterhouse, of New South Wales; K. Goto and T. Naito, of Japan; J. B. Marchionatto, of Argentina; and W. H. Burkholder, Charles Chupp, N. A. Cobb, H. S. Dean, B. O. Dodge, C. W. Dodge, E. W. Erlanson, H. D. House, C. E. Kobuski, G. E. Nichols, F. W. Pennell, F. J. Seaver, C. A. Weatherby, Cynthia Westcott, and R. P. White, of the United States.

³Unpublished records of the Plant Quarantine and Control Administration.

⁴Reference is made by number (italic) to Literature Cited, p. 335.

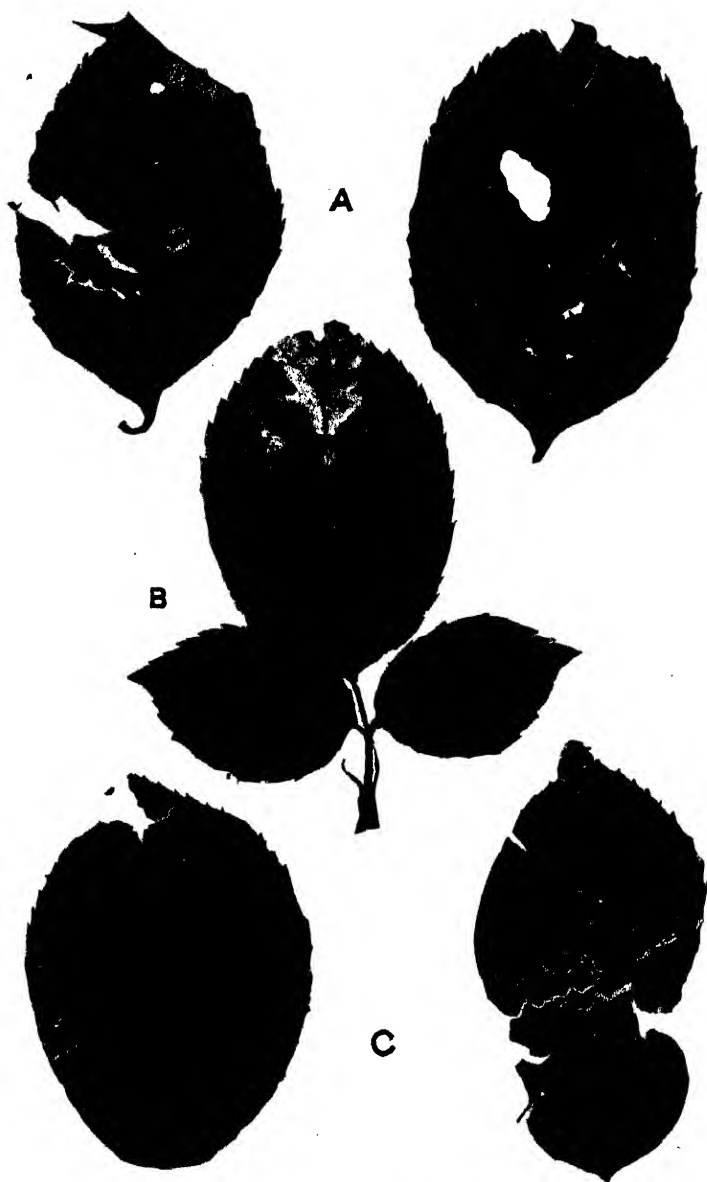


FIGURE 1.—A, Diseased leaves from rose bouquet brought from Germany on the dirigible *Graf Zeppelin* and intercepted by plant-quarantine inspectors at Lakehurst, N. J., October, 1928; B, yellowing in region of anthracnose lesions on leaves of Dora Stoiber rose from Surrey, England, August, 1930; C, diseased leaves of *Rosa chinensis* sent by K. Goto, from Hiroshima, Japan, December, 1926. $\times 1$. Photographs (A) by M. L. F. Foubert, (B) by J. F. Brewer, and (C) by W. R. Fisher



Diseased leaves and stems of *Rosa gentiliana*: a, Lesion of which lower part has fallen away and remainder has become broken. $\times 1$. From Van Fleet rose collection, United States Plant Field Station, Glen Dale, Md., October, 1926. Photograph by M. L. F. Foubert



Diseased stems and leaves of Dr. W. Van Fleet rose: A, Stems; B, D, and E, upper side of leaves; C, lower side of leaves. $\times 1$. Contributed by Westcott, from test gardens of the American Rose Society, at Ithaca, N. Y., August, 1928. Photographs by M. L. F. Foubert

Jacq., *R. gallica* L., *R. gentiliana* Lévl., *R. helenae* Rehder and Wilson, *R. lyoni* Pursh, *R. multiflora* Thunb., *R. nutkana* Presl. (?), *R. nutkana hispida* Fernald, *R. rubiginosa* L., *R. rugosa* Thunb., *R. setigera* Michx., *R. setigera tomentosa* Torr. and Gray, *R. spinosissima* L., *R. sveginzowi* Koehne, *R. wichuraiana* Crép., and *R. woodsi* Lindl. Examples of commercial rose varieties that may be attacked are White Maman Cochet (tea), Dora Stober, Hadley, and Von Scharnhorst (hybrid teas), J. B. Clark (hybrid perpetual), Crimson Globe, Henri Martin (moss), Warburg (Multiflora), Superba (Setigera), Alida Lovett, Climbing American Beauty, Dorothy Perkins, Dr. W. Van Fleet, Eisenach, Excelsa, Purity, Silver Moon, Sodenia, Valentin Beaulieu (hybrid Wichuraianas), Harison's Yellow (Austrian hybrid), and York and Lancaster (damask). Most or all of the roses here named and many others seem to offer little resistance to infection by the pathogene of the disease. Examples of severely diseased growth of *R. chinensis*, *R. gentiliana*, and *Rosa* sp., var. Dr. W. Van Fleet are shown in Figure 1, B, and Plates 1 and 2, respectively. It is notable that in a large rose garden in England, visited by Westcott and the writer in August, 1930, in no case were the ramblers affected, although the disease was prevalent on near-by bush roses—hybrid teas and probably teas.

THE DISEASE

NAME

Cobb (10, *opposite* p. 646), in 1903, published an illustration of a rose leaf affected by the disease discussed herein, labeling it "anthracnose of the rose" (10, *Misc. Pub.* 666, p. 96). In the discussion (10, p. 1063–1065), however, the disease was not called "anthracnose" but "blackspot" and was clearly distinguished from the rose disease caused by *Diplocarpon rosae* Wolf, ordinarily referred to as blackspot, but called by Cobb "star-shaped leaf spot." Although Halsted (15, 16, 17) had previously assigned the name "rose anthracnose" to a rose disease, this disease, as recently explained (25), is apparently of unknown identity. The term as employed by Cobb is here adopted to designate the disease under investigation.

HISTORY AND RANGE

The known range of rose anthracnose includes most European countries, as well as China, Japan (including Chosen), Australia, Africa, South America, Canada, and the United States. Although occurring in practically every continent of the world, the disease, apparently reported heretofore only on rose leaves, has generally escaped notice or has been confused with other rose diseases, particularly blackspot. It has been observed chiefly on the cultivated rose, but it also affects roses growing wild. The only available record of the disease as observed in China and the earliest records of it as observed in Europe and North America concern its occurrence on wild roses.

Passerini's⁵ report of the disease in Italy in 1881, consisting only of a brief characterization of the leaf spot and the fungus associated with it, is the earliest available record of rose anthracnose as a distinct disease. As thus distinguished by Passerini, the disease was later reported in Australia, in New South Wales by Cobb (10, p.

⁵ PASSERINI, G. *HERBARIO CRITTOGAMICO ITALIANO*. Ser. 2, No. 1092. Milan, 1881.

1063-1065) and in Victoria by Grieve (14a); in Japan by Nambū (35); in Russia by Iwanoff (21, p. 100), Jaczewski (22, p. 394), and Siemaszko (40, p. 32); and in Germany by Allescher⁶ (2, p. 13), Diedicke (12, p. 92-93), Flachs (14), and Pape (37a).

As the writer has explained in another paper (27), Italian mycologists seem to have diagnosed this rose disease as bramble (*Rubus*) anthracnose and to have treated these two diseases and others of like nature, including anthracnose of apple (*Malus sylvestris* Mill.) and pear (*Pyrus communis* L.), as one and the same disease. None refer to the report of the disease by Passerini.⁷ The three diseases just mentioned, i. e., anthracnose of rose, of bramble, and of apple and pear, are considered by the writer to be distinct diseases, although they resemble one another closely. Some comparisons of their pathogenes are given in a later section.

Additional records of the disease in Europe, in some cases earlier than those already published, are afforded by mycological specimens from Austria, Czechoslovakia, England, and Germany, and by the presence of anthracnose lesions on phanerogamic specimens from France, Germany, Switzerland, Finland, and Scandinavia, as well as by the diagnosis of the disease on roses from Germany (30), Spain (44), and Ireland,⁸ recently intercepted by plant quarantine inspectors.

The oldest mycological specimens at hand showing leaf spot due to rose anthracnose are those from Austria,⁹ Czechoslovakia (Bohemia),¹⁰ and Germany.¹¹ In the first two the leaf lesions were confused with a leaf spot due to *Septoria*¹² and in the third, apparently, with blackspot.¹³

The phanerogamic specimen from Germany¹⁴ referred to above was collected in the same year as the mycological specimen. The phanerogamic specimens from France, seen in the herbarium of the Royal Botanic Gardens, Kew, England, are of particular interest, as they constitute the earliest known record of the disease. They bear no date but were identified by F. W. Wallroth in 1828 as varieties of "*Rosa scandens*," i. e., "*d. arvensis* B. ovata" and "*d. leucochrea*." In 1902 Briosi (7, p. 313) reported a rose disease from Meaux, France, that is probably rose anthracnose. The disease was observed on garden roses in other parts of France by Burkholder in 1927, and by White and by Dodge in 1930. Dodge states that roses at the National Horticultural School at Versailles were severely diseased. Burkholder in 1930 also observed the disease in Geneva, Switzerland. The occurrence of the disease in Portugal is established by specimens of rose leaves from Portalegre, showing typical anthracnose lesions, recently received through the courtesy of Mathilde Bensaúde.

⁶ Oberammergau, Germany, September, 1901, A. Allescher. SYDOW, H. MYCOTHECA GERMANICA. Fasc. 1, No. 36 (48, p. 519). In the mycological collections of the Bureau of Plant Industry as *Phyllosticta rosarum* Pass.

⁷ Op. cit. (See footnote 5.)

⁸ Op. cit. (See footnote 3.)

⁹ Austria, summer, 1871, F. de Thümen. THÜMEN, F. DE. FUNGI AUSTRIACI. No. 590, Century 6, Dresden, 1872. In the Mycological Collections of the Bureau of Plant Industry as *Septoria rosae* Desm. f. *roseacanthinae* Thümen.

¹⁰ Teplice, Bohemia, autumn, 1873, de Thümen. THÜMEN, F. DE. FUNGI AUSTRIACI. No. 1193, Century 12, Dresden, 1874. In Mycological Collections, Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture, as *Ascochyta rosarum* Lib. f. *centifoliae* Thümen. *Ascochyta rosarum* Lib. = (?) *Septoria rosae* Desm. (38, v. 3, p. 486).

¹¹ Wiesbaden, Germany, Sept. 1873, P. Magnus. Identified as *Asteroma rosae* Lib. (= *Actinonema rosae* (Lib.) Fr. (38, v. 3, p. 408)). Ex herbarium, Charles Peck, Albany State Museum, Albany, N. Y.

¹² Op. cit. (See footnotes 9 and 10.)

¹³ Op. cit. (See footnote 11.)

¹⁴ *Rosa acicularis* Lindl. Münden, Hanover, May 28; July 14, 1873. H. Zabel, 53. In herbarium, Arnold Arboretum, Harvard University, Jamaica Plain, Mass.

Anthracnose lesions are present on a number of phanerogamic specimens of rose from China preserved in the United States and in England; as, for example, on *Rosa brunoni* Lindl. collected by E. H. Wilson, in 1918,¹⁵ and on a specimen of *R. acicularis* Lindl.¹⁶ that he collected in Chosen.

As already stated, rose anthracnose was observed in August, 1930, by Westcott and by the writer in a large rose garden in England. It was also found at Harpenden by Westcott and in a number of other rose plantings in England by the writer. So far as could be learned, the disease had not previously been recognized there as rose anthracnose. Two specimens from that country, however, preserved in the herbarium at Kew, were diagnosed by Wakefield as affected by bramble anthracnose. One of these, dated October, 1926, was from Suffolk County, the other, dated a month later, was from Reading, having been collected there by Buddin, who sent it to Wakefield. At the department of botany, British Museum (Natural History), London, the writer saw one other specimen of anthracnosed rose leaves from England. These had been collected by Ramsbottom at Littlehampton, in 1928. The disease had not been definitely determined, although Ramsbottom had distinguished it from blackspot due to *Diplocarpon rosae*, as others in England had called it.

Relative to the occurrence of rose anthracnose in Africa, Ramsbottom has told the writer that when he was in that country recently he saw roses unmistakably affected by this disease at Cape Town, Union of South Africa; Bulawayo, Southern Rhodesia; and Nairobi, Kenya. The occurrence of the disease in South America was ascertained through Argentine specimens recently sent by Marchionatto. These specimens also served to verify the writer's assumption that the disease which Marchionatto (33, p. 11-13) had earlier reported as possibly Halsted's rose anthracnose is Cobb's rose anthracnose.

The occurrence of rose anthracnose in Canada is established by the presence of lesions on phanerogamic specimens of *Rosa lucida* Ehrh.¹⁷ and *R. humilis* Marsh.¹⁸ from New Brunswick and Quebec, respectively.

In the United States, rose anthracnose is known to occur in Maine, Massachusetts, Connecticut, New York, New Jersey, Pennsylvania, Maryland, Virginia, North Carolina, Florida, Michigan, Texas, and Oregon. Doubtless it is still more widely distributed. In none of the few mycological herbarium specimens available from this country is the leaf spot recognized as that of rose anthracnose. The earliest of these, hitherto undetermined, is from Florida, dated 1893.¹⁹ Other, and in most cases earlier, records are afforded by phanerogamic specimens on which are lesions of the disease. The earliest is dated

¹⁵ Western Szechwan, 1908, E. H. Wilson, 1125. In herbarium, Arnold Arboretum, Harvard University, Jamaica Plain, Mass.

¹⁶ Korea (now Chosen), August, 1917, E. H. Wilson, 8920. In herbarium, Arnold Arboretum, Harvard University, Jamaica Plain, Mass.

¹⁷ Campobello Island, July, 1886, Harry Edwards. In herbarium, New York Botanic Garden, New York.

¹⁸ East Cape, Coffin Island, Aug. 17, 1912, M. L. Fernald, Bayard Long, Harold St. John. No. 7671, FLORA OF THE MAGDALENA ISLANDS, QUEBEC. In Gray herbarium and herbarium of Arnold Arboretum, Harvard University, Cambridge and Jamaica Plain, Mass.

¹⁹ *Rosa* sp. (cult.), Lake City, Fla., November, 1893, P. H. Rolfs. Ex herbarium, Florida Agricultural College, Gainesville, Fla.

1869.²⁰ In 1918 Massey observed the disease on the Dr. W. Van Fleet rose in the test gardens of the Arlington Experiment Farm at Rosslyn, Va., and on this and other roses in the test gardens of the American Rose Society, at Ithaca, N. Y. He diagnosed the disease as bramble anthracnose.

ECONOMIC IMPORTANCE

Although rose anthracnose has been observed chiefly on cultivated roses, little information is available concerning its economic importance. This is due partly to its having been confused with other rose diseases. The writer has not made an extensive survey of rose nurseries in this country to determine whether or not the disease is present in them. It was not found in the eastern nurseries visited in the autumn of 1931. However, in the collection of wild roses in the botanical gardens and arboretum of the University of Michigan, at Ann Arbor, it is considered of such importance that measures have been taken for its control. As observed by Westcott and the writer in the test gardens at Ithaca, N. Y., the disease has not been prevalent during the early part of the growing season, but by late summer it has been present on many varieties, both bedding roses and climbers. Anthracnose lesions were usually found to occur on phanerogamic specimens showing fruit and not on those showing blooms.

Cobb (10, p. 1063) states that "the disease appears at all seasons" in "the greater part of New South Wales." He recently told the writer that it was serious when he was there in 1903, and specimens from Churchward and from Waterhouse, sent at the request of Harrison, show evidence of somewhat the same condition there at present. Nambū (35) states that in Japan the disease may be so severe as to kill the plants, and Marchionatto (33, p. 13) reports that in Argentina it is a veritable enemy of the rose. It is said that the lower leaves on the plant are more severely affected than the upper (10, p. 1064; 35) and that diseased leaves become yellowed (10, p. 1063; 33, p. 12) as well as spotted and may fall away prematurely (33, p. 12). Similar injury ascribed by Heald and Wolf (18, p. 88) to *Cercospora* leaf spot may indicate the presence of rose anthracnose; certainly, lesions of this disease occur on rose leaves²¹ which those investigators diagnosed as affected by the *Cercospora* disease.

SYMPTOMS

LEAF SPOT

In describing the leaf spot, Cobb (10, p. 1063-1064) states that this— is characterized by the appearance on the leaflets of dark spots varying in size but seldom more than a quarter of an inch across, except by the amalgamation of several that have originated near each other. The normal form of the spots is circular, and the first indication of their presence is the loss of the normal green color of the leaflet at the place where the future "Black Spot" will appear.

²⁰ *Rosa carolina* L., Bethlehem, Pa., June, 1869, A. F. Krout. In herbarium, Philadelphia Botanical Club, Philadelphia Academy of Natural Sciences, Philadelphia, Pa.

Rosa virginiana Mill. (originally identified as *R. carolina*), Sandy Hook, N. J., October, 1897, Alex. MacElwee. In herbarium, Philadelphia Botanical Club, Philadelphia Academy of Natural Sciences, Philadelphia, Pa.

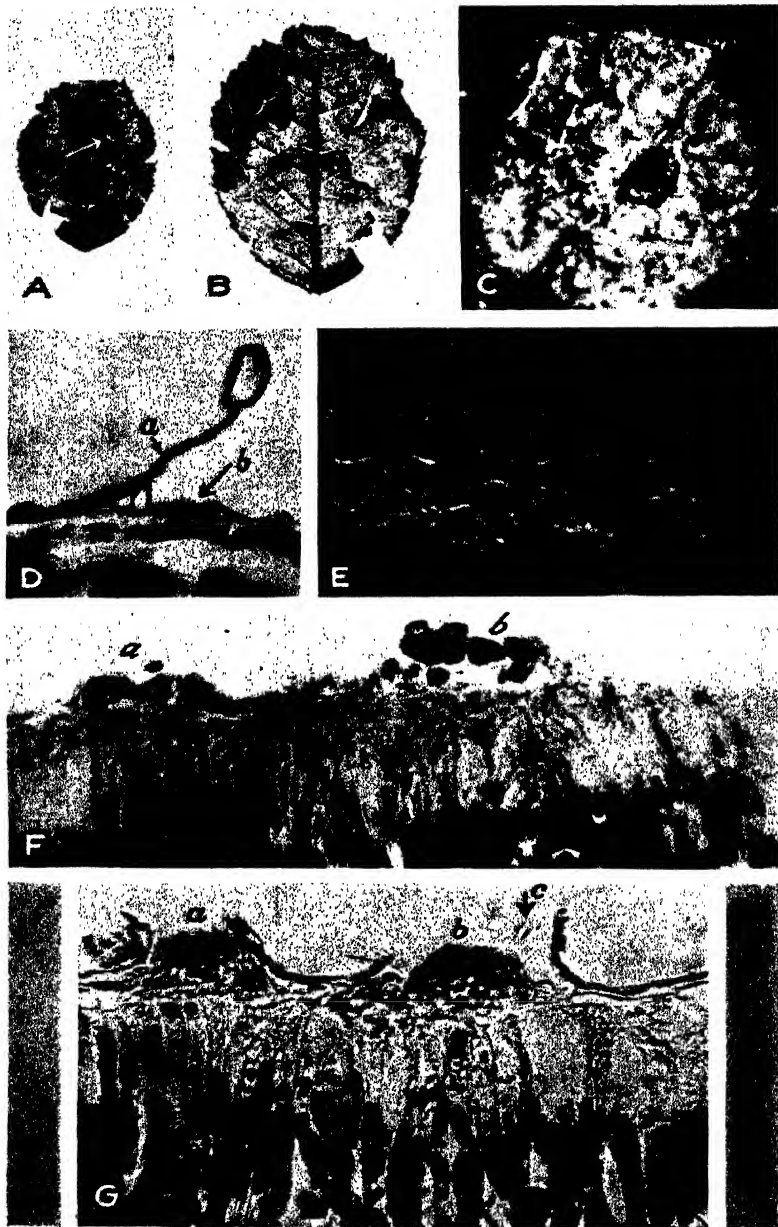
Rosa canina, Jamaica Plain, Mass., Aug. 17, 1888. Ex herbarium, C. and E. Faxon. In Gray herbarium, Harvard University, Cambridge, Mass.

Rosa nutkana? Astoria, Oreg., Aug. 31, 1898, F. V. Coville, 839. In U. S. National Herbarium, Washington, D. C.

²¹ Brenham, Tex., June, 1909, F. D. Heald and F. A. Wolf, 1456 (18, p. 88). In Mycological Collections, Division of Mycology and Disease Survey, Bureau of Plant Industry, U. S. Department of Agriculture.



Sections through anthracnose lesions: A and B, On leaf of Dr. W. Van Fleet rose; C, on stem of *Rosa rubiginosa*; C, a, acervulus. A, $\times 140$; B, $\times 230$; C, $\times 60$. Material from test gardens of the American Rose Society, at Ithaca, N. Y., sent by Westcott, August, 1928. Photomicrographs by M. L. F. Foubert



Anthracnose lesions on leaf of Crimson Globe rose: A, Upper side; B, lower side (\times about $1\frac{1}{2}$); C, enlargement of lesion at A, *a* (\times 22); C, *a*, mass of Coniotheciumlike conidia presumably of the pathogene; D-G, sections of A, *a* (\times 380); D, *a*, ruptured cuticle; D, *b*, and E, *a*, subcuticular hyphae; E, *b*, intraepidermal hypha; E, *c*, spherical bodies from epidermal cells, interpreted as possibly conidia or storage material, or both; F, *b*, Coniotheciumlike conidia, apparently of the pathogene; F, *a*, and G, *a* and *b*, acervuli; G, *c*, conidium, out of focus. Material sent by Chupp from test gardens of the American Rose Society, at Ithaca, N. Y., autumn, 1925. Photographs by J. F. Brewer

* * * From this small beginning the spot increases in size until it reaches the dimensions described above, changing colour meanwhile through dark brown or purple to almost black. Not infrequently a single leaflet bears a score of these spots * * *. At last these spots turn white or at least ash coloured, at the centre, and this is an indication that the fungus which is causing the disease is about to fructify * * *.

Descriptions of the leaf spot by other investigators (22, p. 394; 33, p. 12; 35) agree in all essential particulars with that of Cobb. Marchionatto (33, p. 12) also mentions the yellowing of affected leaves. This discoloration may consist of a mixture of red and yellow, or it may be red shading later to brown, a rather common symptom of the disease. (Fig. 1, B.) The following additional observations of leaf lesions have been made by the writer:

Lesions may occur on any part of the leaf, including midrib and veins (fig. 1; pls. 1 and 2, B-E) and may be thickened as in the closely related disease known as scab of citrus (8, 11) (pl. 3, A and B). They are usually visible on both leaf surfaces. (Pl. 4, A and B.) Lesions are often "dark purplish black" ²² above and are sometimes bordered by a narrow band of "dull livid brown," which is often their color below. Their white or ashen coloration results from a lifting of the cuticle (pl. 4, D, a) or from the etiolation of the leaf tissue (pl. 4, F and G) on the surface where the fungus first gained entrance, usually the upper surface. Lesions may fall away beneath, leaving only a thin papery membrane that ruptures easily (pl. 1, a); they may become perforated at the center (pl. 4, C); or they may fall out entirely, producing the appearance of shot holes. In addition, affected leaves may become distorted and ragged at the margins. (Fig. 1, C, and pl. 2, B.)

STEM CANKER

Stem lesions or cankers (pls. 2, A, and 3, C, and fig 2, A) are considerably smaller than the leaf spot. They are generally circular, or elongate with the longer diameter parallel with the stem axis. Usually they are raised, and sometimes depressed at the center. (Fig. 2, A.) Often they are "dull livid brown," becoming white or ashen at the center. They are seldom more than 2 millimeters across, although by confluence they may be larger and also noticeably

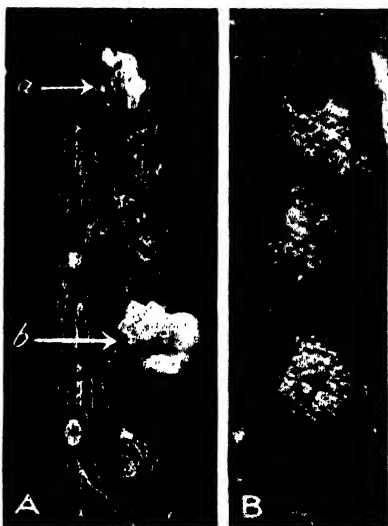


FIGURE 2.—A, Cankers of rose anthracnose on stem of *Rosa rubiginosa*, Ithaca, N. Y. This stem had been disinfected in mercuric chloride (1:1,000), rinsed in sterile water, and kept for 18 days on the surface of a test-tube slant of agar medium, during which period yellowish-green colonies of the pathogene (a and b) developed from two of the lesions, one colony (b) having reached the agar surface. B, Growth of the pathogene from young anthracnose spots on leaf from *Graf Zeppelin* specimen, kept for 5 days in a moist chamber. All $\times 5$. Photographs by M. L. F. Foubert

²² Names of colors in quotation marks are according to the following authority: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

irregular in outline. In general aspect, cankers of this disease, which affects thorns as well as other parts of the cane, are suggestive of initial-infection lesions of brown canker (23, 24), although they do not enlarge later into more or less extensive cankers as do initial-infection lesions of brown canker. Some roses highly susceptible to infection by the pathogene of the former disease, however, are affected little or not at all by the pathogene of the latter. Examples are *Rosa rugosa*, *R. wichuraiana*, and the hybrid *Wichuraianas*, Dr. W. Van Fleet and Silver Moon.



Fig. 111.—Spores of *Phyllosticta Rosarum* Pass., the cause of the Black Spot of the Rose. The simple, smooth, colourless, ellipsoidal spores, measuring $5.72 \times 2.2-3.2\mu$, and averaging $5.5-2.6\mu$, are borne in minute black, nearly spherical perithecia, about 100μ in diameter, which are partly buried in the epidermis of the dead tissue of rose leaves in the midst of more or less circular spots found on the upper surface of the attacked leaves, and characterised by a distinctly concentric arrangement of a striking series of colours, namely, an ash-coloured or nearly white centre, surrounded by brown, purple, and black.



Fig. 112.—Spores of the Black Spot of the Rose, *Phyllosticta rosarum*, obtained from the surface of the leaves, and mounted in water and drawn at once. It will be noted that these spores are smaller than those produced in a water-culture shown in Fig. 111.

FIGURE 3.—Drawings of *Phyllosticta rosarum*, with accompanying legends, as published by Cobb (10) in 1903

LESIONS ON BLOSSOMS

Lesions on the inflorescence observed on hips and pedicels resemble stem lesions; those on calyx lobes, leaf lesions. Petals are probably affected, but lesions on them have not been definitely diagnosed.

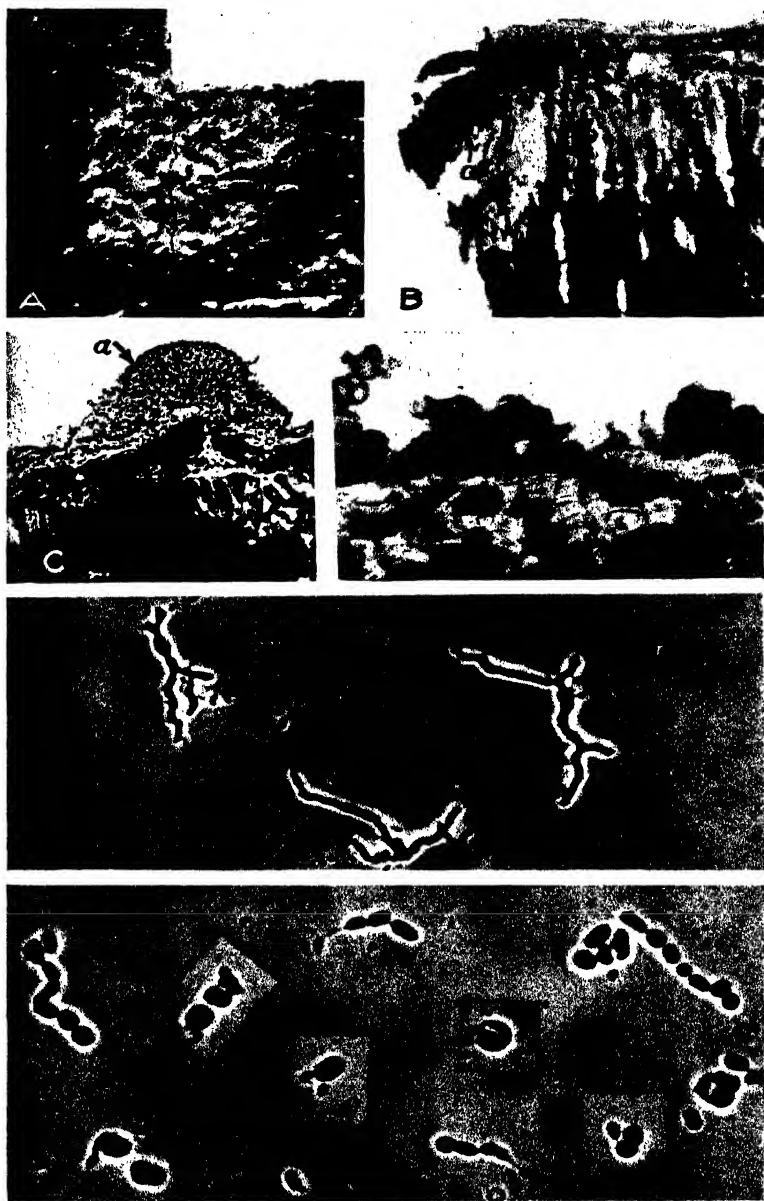
THE FUNGUS

MORPHOLOGY

The perfect stage of the pathogene of rose anthracnose has not been reported. Passerini²³ gives only a brief description of the imperfect stage. In sending leaves of *Rosa chinensis* affected by anthracnose to this country in 1926 (pl. 2, A), K. Goto wrote that the light yellowish-brown acervuli of the pathogene measured from 28μ to 130μ in diameter; the conidiophores, 7.7μ to 14μ by 2.3μ to 3μ ; and the conidia, 5.9μ to 7.4μ by 2.8μ to 5.5μ . For corresponding structures essentially the same measurements were made by Passerini.²³ Cobb (10, legend for Figure 111, here reproduced in Figure 3), Marchionatto (33, p. 13) and Nambū (35) seem to have followed Passerini²³ in

applying to the acervulus of the fungus the term "perithecium," signifying pycnidium. It should also be noted here that Saccardo (39, p. 388) referred to the acervulus in this fungus as a young pycnidium. He did this in citing Lindau's (32, p. 352) reproduction of what is Figure C of Von Tubeuf's drawing as published by Allescher (2, p. 13, 84) (fig. 4); he did not cite the illustrations by Cobb herein mentioned and, in part, reproduced in Figure 3. So far as the writer has been able to determine, the fructification referred to above is an acervulus typical of the form genus *Sphaceloma* (3), which through its further development may become a sporodochium. (Pl. 5, C.) Acervuli may be scarcely visible even when examined under a hand lens (see

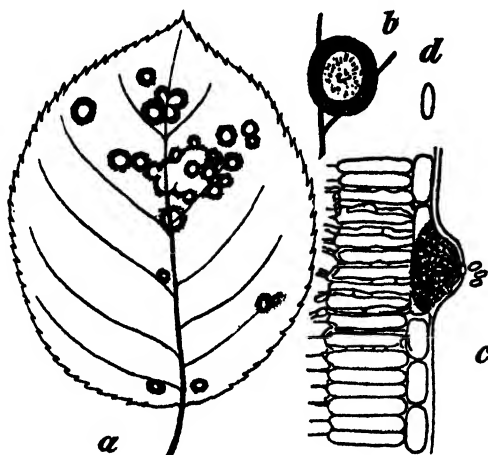
²³ Op. cit. (See footnote 5.)



Pathogene of rose anthracnose. A, Prominent acervull on leaf lesion from specimen collected at Orono, Me., October, 1900, by P. Spaulding (herbarium of P. Spaulding, No. 209). $\times 22$. B, Section of A, a, acervulus; b, conidiophores still confined to epidermal cells in which they grew. $\times 380$. C, Section of a leaf lesion shown in Figure 3, A; a, sporodochial growth of the fungus; b, hyphae passing from lower part of sporodochium, between and beneath epidermal cells. $\times 200$. D, Section corresponding to Plate 4, F, showing additional conotheciumlike conidia, presumably of the rose-anthracnose fungus. $\times 380$. E and F, 7-day-old water cultures from conidia sown in drops of water on glass slides, kept at room temperature and now germinated (E) or in a refrigerator and now greatly swollen (F). $\times 380$. Photographs by M. L. F. Foubert

pl. 4, C), but they may be fairly prominent (pl. 5, A, and fig. 5) and somewhat concentrically arranged (fig. 5).

Plates 4 and 5 show on rose leaves subcuticular (pl. 4, D, *b*, and E, *a*) and intraepidermal hyaline hyphae (pl. 4, E, *b*), colored acervuli (pl. 4, F, *a*, and G, *a* and *b*, and pl. 5, B, *a* and *b*), and Coniothecium-like conidia presumed to be those of the pathogene (pl. 4, F, *b*, and pl. 5, D), as well as spherical granules (pl. 4, E, *c*) interpreted as possibly conidia or storage substances, or both, produced in the epidermal cells shown in Plate 4, E. There are also shown germinated (pl. 5, E) and greatly swollen (pl. 5, F) conidia producing sprout conidia. These represent 7-day-old water cultures kept at room temperature



Phyllosticta Rosarum Passer. *a*. Ein Blättchen mit dem Pilze in natürlicher Grösse. *b*. Ein einzelner Flecken etwas vergrössert. *c*. Ein Fruchthäuse noch von der Epidermis bedeckt, von der Seite, mit einigen ausgetretenen Sporen. *d*. Eine einzelne sehr vergrösserte Spore. *b* u. *c* stark, *d* noch mehr vergrössert. Nach der Natur von Dr. v. Tubeuf gezeichnet.

FIGURE 4.—Von Tubeuf's drawings of *Phyllosticta rosarum*, with accompanying legend, as published by Allescher (*l. p. 15, 84*) in 1898

(pl. 5, E) and in a refrigerator (pl. 5, F). The germinated and greatly swollen conidia are those originally sown in these cultures.

NAME, CLASSIFICATION, AND IDENTITY

Passerini²⁴ described as *Phyllosticta rosarum* the pathogene of the disease to which Cobb later applied the term "rose anthracnose." Passerini's description of the fungus was based on leaves of the cultivated rose collected at Vigheffio, near Parma, Italy.²⁴ The writer has seen these specimens at the laboratories of cryptogamic botany, Harvard University, Cambridge, Mass.; the New York Botanic Garden, New York, N. Y. (fig. 6); the department of botany, British Museum (Natural History), London, England; and the botanical

²⁴ Op. cit. (See footnote 5.)

department, University of Edinburgh, Edinburgh, Scotland. In all cases the leaf spot is typical of rose anthracnose. A fragment of the specimen at the New York Botanic Garden, obtained for microscopic comparison, showed fruiting bodies identical with those here illustrated.

As previously stated, the pathogene of this disease has not only been identified as *Phyllosticta rosarum* but has also been treated (27) as identical with *Elsinoe veneta* (Speg.) Jenkins, which causes bramble anthracnose, and with *E. piri* (Wor.) Jenkins, which causes apple and pear anthracnose. For the present, however, these three organisms

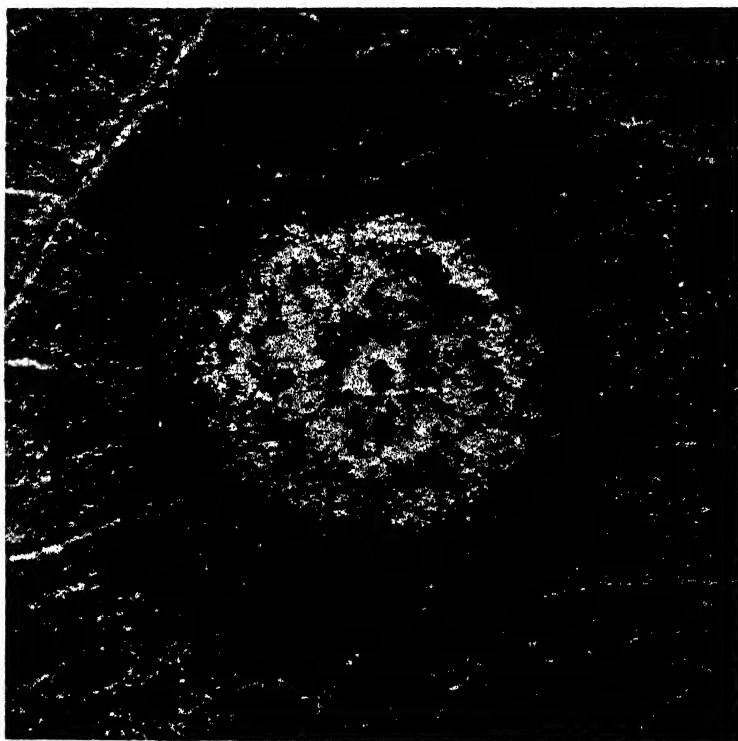


FIGURE 5.—Pathogene of rose anthracnose. Acervuli in more or less concentric arrangement on upper surface of lesion on Silver Moon rose. From test gardens of the American Rose Society, at Ithaca, N. Y., August, 1931. X about 20. Photograph by M. L. F. Foubert

are treated as distinct, as are the three anthracnose diseases that they cause. Since no perfect stage of the rose fungus has yet been found and since its imperfect stage as known is typical of *Sphaceloma* rather than of *Phyllosticta*, the fungus is here transferred to the former genus as *Sphaceloma rosarum* (Pass.), n. comb.

In several monographic treatments of the genus *Phyllosticta* (2; 32; 38, v. 10, p. 100-138) the species under discussion is included, and in one (2, p. 84) it is employed to illustrate the characteristics of that genus. Moreover *Phyllosticta rosarum*, i. e., *Sphaceloma rosarum*,

has been treated as identical with *Phyllosticta rosae* Desm. (29, p. 416) by Von Höhnelt (19, p. 135-136), but this is evidently another fungus. Von Höhnelt (19, p. 136) has also expressed the opinion that Passerini's type material, referred to in the previous section, is actually *Phragmidium subcorticinum* (Schrank) Wint. This is not surprising, since

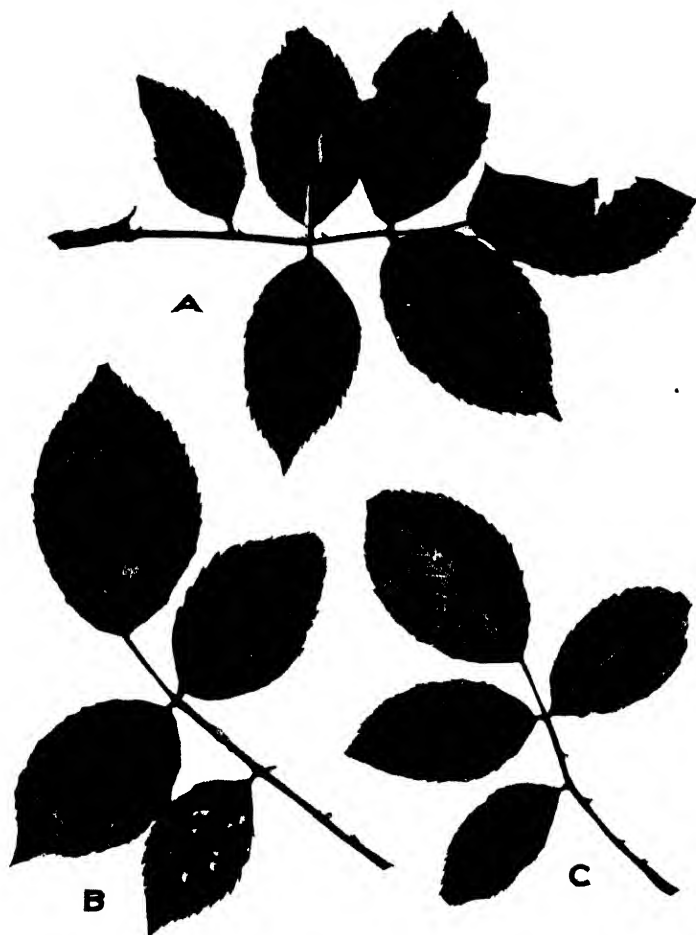


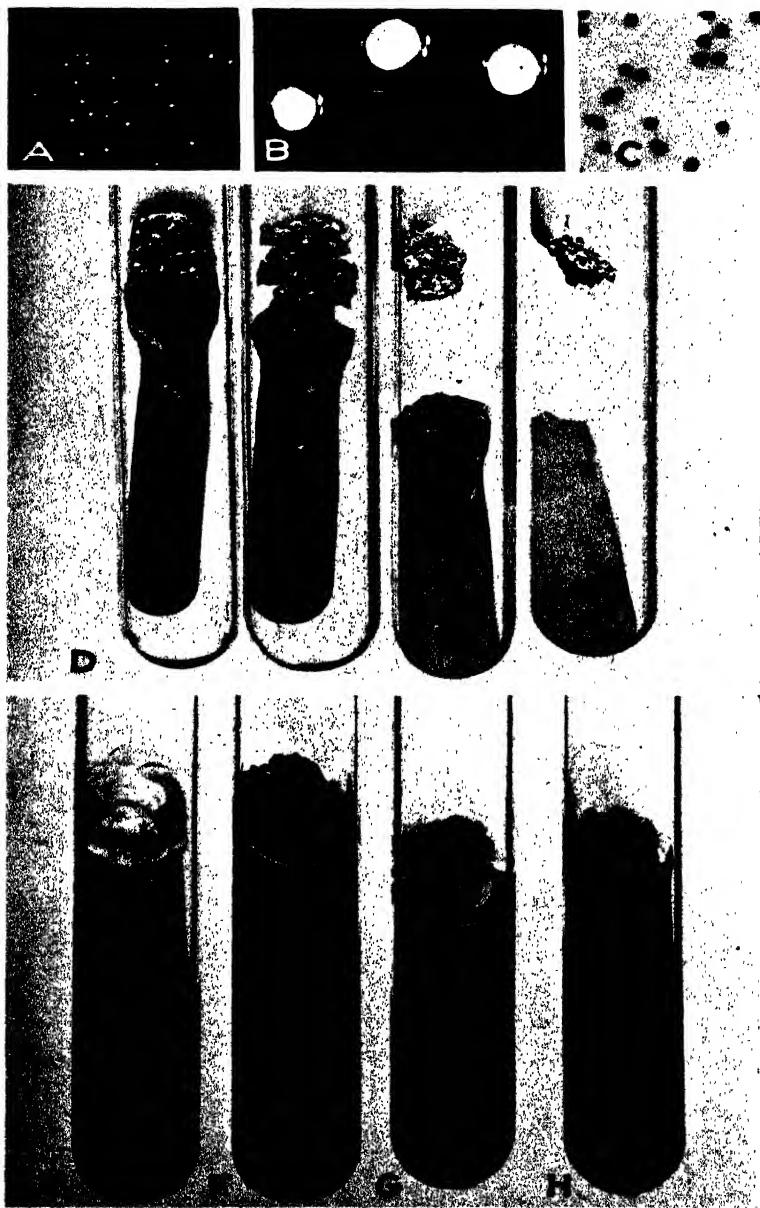
FIGURE 6.—*Phyllosticta rosarum* on leaves of cultivated rose collected by Passerini at Vigheffo, Italy. (PASSERINI, G. ERBARIO CRITTOGAMICO ITALIANO, Ser. 2, No. 1092. Milan, 1881.) Ex herbarium, New York Botanic Garden. A and B, Upper leaf surface; C, lower leaf surface. $\times 1$. Photograph contributed by F. J. Seaver

purple leaf lesions due to rose rust may resemble closely those of rose anthracnose, although the readily recognized rust pustules serve to distinguish the two diseases. Ciferri's (9, p. 40) report of *Phyllosticta rosarum* on rose apparently relates to some other rose fungus. *Sphaceloma rosarum* has been confused with still other rose fungi, including *Diplocarpon rosae*, as already mentioned.

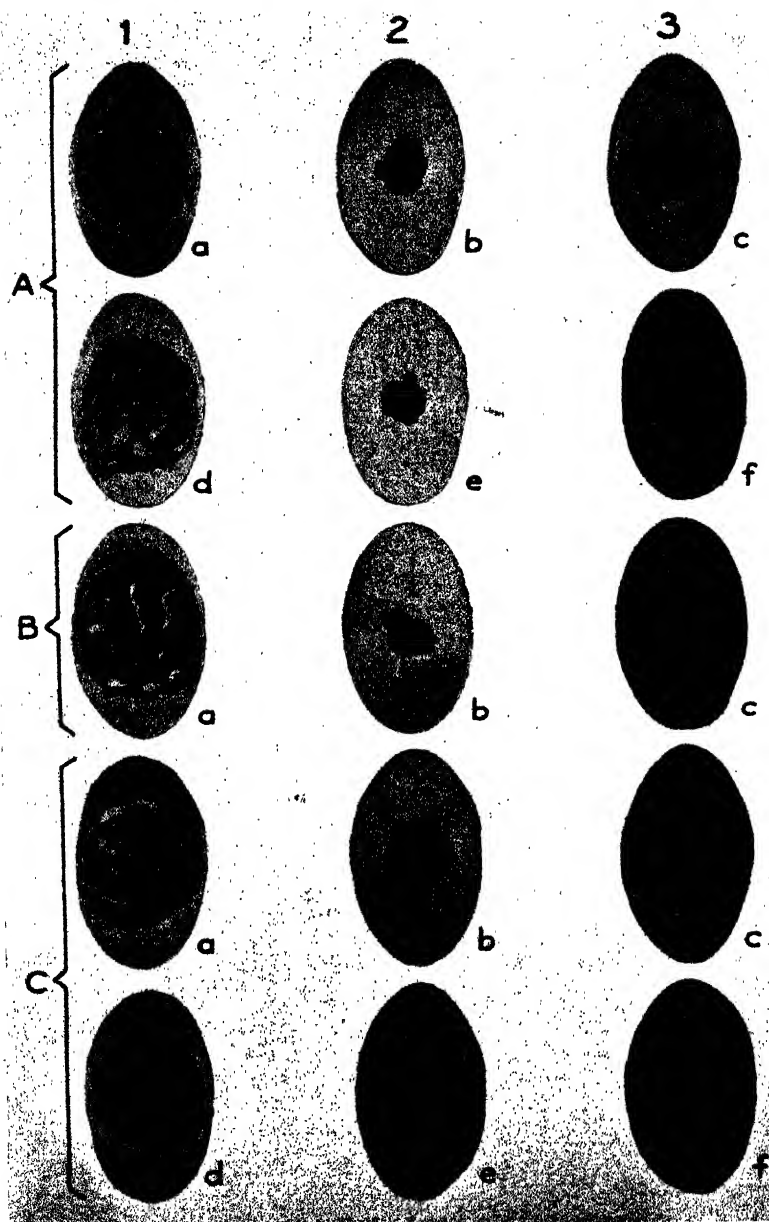
ISOLATIONS AND CULTURAL CHARACTERISTICS

Cultures of *Sphaceloma rosarum* on potato-dextrose agar (pl. 6, A-C) represent platings from conidia developed on the *Graf Zeppelin* specimen. (Fig. 2, B.) The week-old colonies (pl. 6, A and B) were grown at room temperature, and the 3-weeks-old colonies (C) were grown in a refrigerator. The younger colonies were just becoming reddish; the older, viewed through the reverse of the Petri dish, were "liver brown," verging on "black." Individual colonies from the week-old cultures were transferred to test-tube slants of potato-dextrose agar, one colony to each test tube. At 4 weeks of age these cultures resembled that shown in Plate 6, H, which represents a subculture from this set of cultures. Their coloration was then a mixture of "gray" to "dull purplish black" at the center, bordered by "maroon," with the surrounding medium "light vinaceous cinnamon." Typical cultures selected after four months are shown in Plate 6, D. They were then "fuscous black" mingled with "olivaceous black" with the deep crevices and bordering parts, as well as the surrounding medium, "bone-brown." The 5-weeks-old parallel cultures shown in Plate 6, E-H, represent the following isolations: H, a subculture from the set of cultures shown in Plate 6, D; F, a subculture from one of the colonies shown in Figure 2, A; E, an isolation from the Dr. W. Van Fleet rose, made in 1923 by Massey (28, footnote 2); and G, an isolation from the same variety made in 1928 by the writer. The culture shown in Plate 6, E, represents the pulvinate type of growth in this fungus and the cultures shown at D and at F to H, represent the convolute type. The occurrence of these types of growth in the genus *Sphaceloma* has been discussed in a previous paper (26), with the explanation that they have been observed in cultures on carrot, Molisch's, and potato-dextrose-agar media.

Cultures of *Sphaceloma rosarum*, *Elsinoe piri*, and *E. veneta* grown under similar conditions are shown in Plate 7. Vertical columns 1, 2, and 3 represent, respectively, potato-dextrose, beef, and corn-meal agar media. These cultures were grown at room temperature and for the most part in the dark. Plate 7 shows the pulvinate type of growth in *S. rosarum* (A, d-f), in *E. piri* (B, a-c), and in *E. veneta* (C, a-c). The convolute type in *E. piri* is not included in this comparison, but, allowing for slight differences, would resemble that of *S. rosarum* (A, a-c) and *E. veneta* (C, d-f). It will be observed that on the three media here represented any one isolation is distinguishable from the others and that those of the same type of growth resemble each other closely. In other sets of parallel cultures the isolations have varied somewhat in appearance from those here shown, but in each set the individual isolations have been separable; and thus far, whether convolute or pulvinate, each has maintained its characteristic type of growth. On potato-dextrose agar the convolute cultures were much darker and of a more uniform color, in general "dull purplish black," than the pulvinate cultures, which were variegated and lighter in tone. The cultures on beef agar were of a brownish coloration except that shown in A, e, which was noticeably lighter than the others. The yellowish-green chromogenesis reported by Osterwalder (36) in *E. piri* had been seen in this species as grown, for example, on corn-meal, glycerin, and potato-dextrose agar media. The same chromogenesis occurs in the isolation from



Sphaceloma rosarum on potato-dextrose-agar medium. A-C, Petri-dish cultures from conidia; A, week-old colonies grown at room temperature; B, enlargement of a few colonies in A, to show mucilaginous covering; C, 3-week-old colonies grown in a refrigerator. D, 4-months-old test-tube slant cultures, each inoculated with a colony from culture shown in A. E-H, 5-weeks-old cultures, each representing a different isolation; E, pulvinate type of growth; D, F-H, convolute type of growth. A-D, Growth from conidia from *Graf Zeppelin* specimen (fig. 3, B); E and G, isolations from Dr. W. Van Fleet rose, made in 1923 by Massey (E) and in 1928 by the writer (G); F and H, subcultures from isolations shown in Figure 3, A, and Plate 6, D, respectively. Photographs by M. L. F. Foubert



Parallel cultures of *Sphaceloma rosarum* (A, a-f), *Elsinoe piri* (B, a-c), and *F. veneta* (C, a-f) on test-tube slants of potato-dextrose (vertical column 1), beef (vertical column 2), and corn-meal (vertical column 3) agar media. A, a-c, and C, d-f.—Convolute type of growth, and A, d-f, B, a-c, and C, a-c, pulvinate type of growth, as determined particularly by the character of the colony on potato-dextrose agar. A, a-c.—Isolation from *Rosa gentiana* (pl. 1); A, d-f, same isolation as that shown in Plate 6, E. B, a-c.—Isolation by Osterwalder (35), in 1926, from Jonathan apple grown in Switzerland. C, a-c.—Isolation by L. K. Jones, in 1923, from *Rubus occidentalis* L., Wisconsin. C, d-f.—Isolation by the writer, in 1925, from *R. neglectus* Peck, Virginia. Photograph of color drawings by J. Marion Shull

Crimson Globe and in a few other isolations, from both rose and brambles, made by the writer. As a rule, however, the coloration imparted to the media by these organisms is a pinkish or reddish hue, sometimes "madder-red." This coloration was present to a greater or less degree throughout the media in all the cultures shown in Plate 7, vertical column 3, where the crescent-shaped discoloration beneath the colonies in B, c; C, c and f, is also due to this chromogenesis. As is often the case with old cultures of *Sphaceloma*, conidia are probably absent or sparse in all or most of the cultures shown in Plates 6 and 7. They were abundant in the cultures illustrated in Figure 2, A, a and b, and B.

In young cultures grown in parallel, conidia of *Elsinoe piri* were definitely smaller than those of *E. veneta*. No conidia could be found in a culture of *Sphaceloma rosarum* included in the same comparison. Harris, comparing conidia of this species from a rose stem canker with those of *E. veneta* taken directly from its bramble host, found them of nearly the same size; i. e., those of the rose fungus measured 5.9μ by 3.1μ and those of the bramble, 6.5μ by 3.0μ , while cultures of the two were practically indistinguishable. The material of the rose fungus studied by Harris was taken from that sent to Wakefield by Buddin in 1926 (p. 65). This culture, not now living, was isolated by Wakefield; and the other culture, from brambles in England, was made by R. C. Harris. The conidia of *E. piri* referred to were from the isolation of this fungus from the Portuguese specimens of diseased apples contributed by Bensaúde (27); those of the parallel culture of *E. veneta*, from the isolation of that fungus shown in Plate 1, C, d-f.

ASSOCIATED FUNGI

Associated with *Sphaceloma rosarum*, there has been found an *Alternaria*, as first noted by Massey and later by the writer, and also a yeastlike imperfect fungus and an ascomycete. The yeastlike fungus is probably similar to that which Stoneman (41, p. 77) found associated with *Gloeosporium venetum*, if not identical with it, and is possibly to be classified in the genus *Phymatotrichum* (5, p. 116) as typified by *P. gemellum* Bon. (5, p. 116, fig. 138), although not in this genus as emended by Duggar (13). It is certainly of the same form genus as *P. vaccarum* Oud. (37, p. 392-394, figs. 7-10) and various similar fungi, including a fungus apparently causing a rose blight, reported by the present writer as *Polyspora* ? sp.²⁵ and a "Dematium-like" fungus said to cause a blight of cherry (*Prunus*) (10, Misc. Pub. 666, p. 109-112, figs. 122, a; 123, a-d; 124; 125). The ascomycete on one occasion found in association with *S. rosarum* is identified as *Pringsheimia sepincola* (Fr.) Höhn. (20, p. 97). It may be noted that according to the synonymy given in the reference just cited *Sphaerulina intermixta* Berk. and Br. should be the same fungus. The writer was unable to demonstrate for the former ascomycete the "*Dematium pullulans*" which Brefeld (6, pl. 7, figs. 47-50) claimed that he obtained when he grew cultures of the latter from ascospores.

²⁵ MARTIN, G. H. DISEASES OF FOREST AND SHADE TREES, ORNAMENTAL AND MISCELLANEOUS PLANTS IN THE UNITED STATES IN 1924. U. S. Dept. Agr., Bur. Plant Indus. Plant Disease Rptr. Sup. 42 (p. 362), 380 p. 1925. [Mimeographed.]

PATHOGENICITY

That *Sphaceloma rosarum* is the cause of rose anthracnose was proved by artificial inoculations made under greenhouse conditions at Ithaca, N. Y., on March 15, 1927. The plants inoculated were the Dr. W. Van Fleet rose, propagated in a greenhouse at Washington, D. C., by the Division of Gardens and Grounds, Bureau of Plant Industry, United States Department of Agriculture. The inoculum was a several-weeks-old potato-dextrose-agar culture of the isolation from *Rosa gentiliana* shown in Plate 7, A, a-c. Small masses of the culture, crushed and moistened in water, were forced between the closely folded halves of young leaflets, so that the fungus came in contact only or mostly with the upper leaf surface. Each of five leaflets so inoculated was wrapped in small pieces of cotton saturated with water; then the entire leaf, together with part of the stem from which it grew, was loosely wrapped in waxed paper. The coverings were removed after 48 hours. Except for the absence of inoculum, the five leaflets serving as checks were treated in the same manner as those inoculated.

On March 27, reddish lesions less than a millimeter in diameter had developed on the upper surface of the leaflets inoculated. These lesions when examined on April 2, had enlarged somewhat; a few showed whitish centers, and some had become visible also on the lower surface. Microscopic examination revealed the presence of the fungus in the lesions, and in reisolation cultures only growth of this fungus developed. The cultures were made by disinfecting in mercuric chloride (1:1,000) entire leaflets or parts of leaflets bearing lesions, rinsing them in sterile water, and transferring them to potato-dextrose-agar slants.

The rose plants used in the experiment just described were later inoculated with a culture of *Elsinoe veneta*, but in this instance there was no evidence of infection. Purplish blotches appeared within a day or so on the inoculated leaves but were not typical of rose anthracnose, and no organism was reisolated from them. Essentially the same results were obtained when, likewise under greenhouse conditions, tender leaves of young seedling apple trees were inoculated with a cultural growth of *E. veneta*, as well as of *Sphaceloma* from unrelated hosts. The inoculated leaves were left on the trees, and after a time the discoloration entirely disappeared. In this connection Alexander's (1, p. 71, 72) inoculations with *Gloeosporium venetum* Speg., i. e., *E. veneta*, should be noted. He states (1, p. 71) that under "very * * * humid conditions" this organism "produced a slight lesion on *Opuntia inermis* after 48 hours," and (1, p. 72) that it produced a "considerable amount of rot" on apple fruits "within 24 hours." He does not report reisolations from either pricklypear or apple, nor does he explain whether this decay occurred on the apples inoculated by inserting the pure culture into the tissue with a needle or on those sprayed with conidia, or on both.

CONTROL

The only control measures known to have been reported for rose anthracnose are those by Cobb (10, p. 1064-1065) and Marchionatto (33, p. 13). The former advises the removal and destruction of affected parts and, where the disease is severe, spraying with Bordeaux

mixture; the latter recommends spraying with this fungicide or with ammoniacal copper carbonate as the disease appears. From Naito it was learned that in Japan the disease is controlled by spraying with Bordeaux mixture. During the last few years, Erlanson, following the suggestion of the present writer, controlled the disease in the wild-rose garden at the University of Michigan botanical gardens and arboretum at Ann Arbor by spraying the roses with lime-sulphur before the leaf buds opened and with Bordeaux mixture after the leaves had developed. This is an adaptation of the recommendations given by Bennett (4) for the control of bramble anthracnose in Michigan.

SUMMARY

Rose anthracnose has been confused with a number of other rose diseases, and for this reason has not been generally recognized as a distinct disease. It is of considerable economic importance, affecting many rose species and varieties and including in its range the United States, Canada, Argentina, Japan, China, New South Wales, most European countries, and Africa. Its occurrence in France in 1828 and in the United States in 1869 is reported. The pathogene, *Phyllosticta rosarum*, described by Passerini on the basis of material that he collected in Italy in 1881, is here transferred to the genus *Sphaceloma* as *S. rosarum* (Pass.), n. comb. Morphological and cultural characteristics of the fungus are given, together with proof of its being the cause of rose anthracnose. Fungi associated with *S. rosarum* are mentioned, symptoms of the disease described, and the available information pertaining to its control is summarized.

LITERATURE CITED

- (1) ALEXANDER, W. B.
1925. NATURAL ENEMIES OF PRICKLY PEAR AND THEIR INTRODUCTION INTO AUSTRALIA. Aust. Inst. Sci. and Indus. Bul. 29, 80 p., illus.
- (2) ALLESCHER, A.
1898. FUNGI IMPERFECTI: PHYLLOSTICTA PERS. In Rabenhorst's Kryptogamen-Flora Aufl. 2, Abt. 6, p. 12-167, illus. Leipzig.
- (3) BARY, A. DE.
1874. ÜBER DEN SOGENANTEN BRENNEN (PECH) DER REBEN. Ann. Oenol. 4:165-167.
- (4) BENNETT, C. W.
1928. MICHIGAN RASPBERRY DISEASES. Mich. Agr. Expt. Sta. Spec. Bul. 178, 52 p., illus.
- (5) BONORDEN, H. F.
1851. HANDBUCH DER ALLGEMEINEN MYKOLOGIE ALS ANLEITUNG ZUM STUDIUM DERSELBEN, NEBST SPEZIELLEN BEITRÄGEN ZUR VERVOLLKOMMUNG DIESES ZWEIGES DER NATURKUNDE. 336 p., illus. Stuttgart.
- (6) BREFELD, O.
1891. UNTERSUCHUNGEN AUS DEM GESAMTGEBIETE DER MYKOLOGIE, FORTSETZUNG DER SCHIMMEL- UND HEFENPILZE. Heft 10, 378 p. Ascomyceten II, illus. Münster.
- (7) BRIOSI, G.
1902. RASSEGNA CRITTOGAMICA DEI MESI DI MARZO A LUGLIO 1900. Atti Ist. Bot. R. Univ. Pavia (2) 7:[295]-316.
- (8) BUTLER, E. J.
1930. SOME ASPECTS OF THE MORBID ANATOMY OF PLANTS. Ann. Appl. Biol. 17: 175-212, illus.
- (9) CIFERRI, R.
1922. NOTAE MYCOLOGICAE ET PHYTOPATHOLOGICAE. SERIE 1, NO. 1-11. Ann. Mycol. 20:34-53, illus.

- (10) COBB, N. A.
1903-4. LETTERS ON THE DISEASES OF PLANTS. *Agr. Gaz. N. S. Wales* 14:627-652; 681-712; 955-986; 1057-1072, illus., 1903; 15:1-19, illus., 1904. [Also published as *Dept. Agr. N. S. Wales Misc. Pub.* 666, 133 p., illus. 1904.]
- (11) CUNNINGHAM, H. S.
1928. THE HISTOLOGY OF LESIONS PRODUCED BY SPHACELOMA FAWCETTII JENKINS ON LEAVES OF CITRUS. *Phytopathology* 18: 539-545, illus.
- (12) DIEDICKE, H.
1915. FUNGI IMPERFECTI: PHYLLOSTICTA PERS. *In* *Kryptogamenflora der Mark Brandenburg*, v. 9, p. 14-110, illus. Leipzig.
- (13) DUGGAR, B. M.
1916. THE TEXAS ROOT ROT FUNGUS AND ITS CONIDIAL STAGE. *Ann. Missouri Bot. Gard.* 3:11-23, illus.
- (14) FLACHS, K.
1931. KRANKHEITEN UND PARASITEN DER ZIERPFLANZEN; EIN BESTIMMUNGS- UND NACHSCHLAGEBUCH FÜR BIOLOGEN, PFLANZENÄRZTE, GÄRTNER UND GARTENFREUNDE. 558 p., illus. Stuttgart.
- (14a) GRIEVE, B. J.
1932. ROSE DISEASES AND THEIR CONTROL. XIV. ROSE LEAF SCORCH. *Jour. Dept. Agr. Victoria* 30: 92-94, illus.
- (15) HALSTED, B. D.
1893. FUNGOUS TROUBLES OF ROSES. *N. J. Agr. Expt. Sta. Rpt.* (1892) 13:280-283.
- (16) ———
1894. THE ANTHRACNOSE OF THE ROSE. *N. J. Agr. Expt. Sta. Rpt.* (1893) 14:401-405, illus.
- (17) ———
1895. SOME OF THE FUNGOUS DISEASES OF ORNAMENTAL PLANTS. *N. J. Agr. Expt. Sta. Rpt.* (1894) 15:362-386, illus.
- (18) HEALD, F. D., and WOLF, F. A.
1912. A PLANT DISEASE SURVEY IN THE VICINITY OF SAN ANTONIO, TEXAS. *U. S. Dept. Agr., Bur. Plant Indus. Bul.* 226, 129 p., illus.
- (19) HÖHNEL, F. v.
1919. FUNGI IMPERFECTI. BEITRÄGE ZUR KENNTNIS DERSELBE. *Hedwigia* 60:129-209.
- (20) ———
1920. MYKOLOGISCHE FRAGMENTE. *Ann. Mycol.* 18:[71]-97.
- (21) IWANOFF, K. S.
1900. DIE IM SOMMER 1898 BEI PETERSBURG (RUSSLAND) BEOBSACHTEN KRANKHEITEN. *Ztschr. Pflanzenkrank.* 10:97-102.
- (22) JACZEWSKI, A. A.
1911-12. YEAR BOOK OF INFORMATION ON THE DISEASES OF PLANTS. *Russia-mikologii i Fitopatologii* v. 7 and 8, 463 p., illus.
- (23) JENKINS, A. E.
1927. BROWN CANCER OF THE ROSE. *Amer. Rose Ann.* 1927:161-182, illus.
- (24) ———
1931. DEVELOPMENT OF BROWN CANCER OF ROSES. *Jour. Agr. Research* 42:293-299, illus.
- (25) ———
1931. GLOEOSPORIUM ROSAE, A NOMEN NUDUM. *Mycologia* 23:223-224.
- (26) ———
1931. DEVELOPMENT OF THE CITRUS-SCAB ORGANISM, SPHACELOMA FAWCETTII. *Jour. Agr. Research* 42:545-558, illus.
- (27) ———
1932. ELSINOE ON APPLE AND PEAR. *Jour. Agr. Research* 44:689-700, illus.
- (28) ——— and HORSFALL, J. G.
1929. A COMPARISON OF TWO SPECIES OF PLECTODISCELLA. *Mycologia* 21:44-51, illus.
- (29) KICKX, J.
1867. FLORE CRYPTOGAMIQUE DES FLANDRES . . . v. 1. 521 p. Gand.

- (30) KISLIUK, M., JR.
1929. AIR ROUTES, GERMAN DIRIGIBLE "GRAF ZEPPELIN" AND PLANT QUARANTINES. *Ent. News* 40:196-197.
- (31) ———
1929. PLANT QUARANTINE INSPECTION OF THE DIRIGIBLE "GRAF ZEPPELIN." (Scientific Note) *Jour. Econ. Ent.* 22:594-595.
- (32) LINDAU, G.
1900. SPHAEROPSIDALES. In Engler, A., and Prantl, K. *Die Natürlichen Pflanzenfamilien.* 1, Abt. 1, p. 351-352, illus.
- (33) MARCHIONATTO, J. B.
1924. NUEVA CONTRIBUCIÓN AL CONOCIMIENTO DE LOS HONGOS PARÁSITOS DE LAS PLANTAS CULTIVADAS. *Rev. Facult. Agron. La Plata* 15 (3): [7]-21, illus.
- (34) MARLATT, C. L.
1929. REPORT OF THE CHIEF OF THE PLANT QUARANTINE AND CONTROL ADMINISTRATION. [U. S. Dept. Agr.] 72 p. Washington, D. C.
- (35) NAMBU, N.
1915. PHYLLOSTICTA ROSARUM PASS. *Jour. Plant Protect.* [Tokyo] 2:192.
- (36) OSTERWALDER, A.
1926. DIE FLECKENBILDUNG BEIM JONATHAN APFEL (JONATHAN SPOT). *Ztschr. Pflanzenkrankh.* 36:264-269, illus.
- (37) OUDEMANS, C. A. J. A.
1901. CONTRIBUTIONS TO THE KNOWLEDGE OF SOME UNDESCRIBED OR IMPERFECTLY KNOWN FUNGI (4th part and end). *Melanconiceae.* *Roy. Acad. Sci. Amsterdam Sect. Sci. Proc.* 3:386-400, illus.
- (37a) PAPE, H.
1932. DIE PRAXIS DER BEKÄMPFUNG VON KRANKHEITEN UND SCHÄDLINGEN DER ZIERPFLANZEN. 361 p., illus. Berlin.
- (38) SACCARDO, P. A.
1884-92. SYLLOGE FUNGORUM . . . v. 3, 1884; v. 10, 1892. Patavii.
- (39) ———
1911. INDEX ICONUM FUNGORUM. In *Sylloge Fungorum.* v. 20. Patavii.
- (40) SIEMASZKO, V.
1915. [CONTRIBUTIONS TOWARDS A MYCOLOGICAL FLORA OF THE DISTRICT OF SUCHUM.] *Mater. Mikol. i Fitopat. Rosii Biuro Mikol. i Fitopat.* 1 (3): 23-41, illus.
- (41) STONEMAN, B.
1898. A COMPARATIVE STUDY OF THE DEVELOPMENT OF SOME ANTHRACNOSES. *Bot. Gaz.* 26:69-120, illus.
- (42) SYDOW, H.
1903. MYCOTHECA GERMANICA FASC. 1 (NO. 1-50). *Ann. Mycol.* 1:[519]-521.
- (43) UNITED STATES DEPARTMENT OF AGRICULTURE, PLANT QUARANTINE AND CONTROL ADMINISTRATION.
1929. PESTS COLLECTED FROM IMPORTED PLANTS AND PLANT PRODUCTS FROM JANUARY 1 TO DECEMBER 31, 1928, INCLUSIVE. U. S. Dept. Agr. Serv. and Regulat. Announc. Ann. Letter Inform. 41:157-234.
- (44) ———
1930. LIST OF PESTS INTERCEPTED ON IMPORTED PLANTS AND PLANT PRODUCTS DURING THE CALENDAR YEAR 1929. U. S. Dept. Agr. Serv. and Regulat. Announc. Sup. p. 249-331.

A BACTERIAL DISEASE OF THE TUNG-OIL TREE¹

By LUCIA McCULLOCH, *Associate Pathologist*, and J. B. DEMAREE, *Pathologist*,
Division of Horticultural Crops and Diseases, Bureau of Plant Industry, United
States Department of Agriculture

INTRODUCTION

A bacterial leaf spot of the tung-oil or wood-oil tree (*Aleurites fordii* Hemsl.) was observed by Boyd² in several localities in southern Georgia in 1929. The first specimens of diseased leaves received by the senior writer were collected by Doctor Boyd October 25, 1929, from a nursery in southwestern Georgia. Infected leaves of the same season's growth were collected later by the junior writer. Numerous petiole and stem lesions on material from Georgia have also been examined, but so far no microscopic evidence of bacteria has been found in these, and efforts to isolate bacteria have been unsuccessful.

In 1930 and 1931 this bacterial disease was either absent or so slight as to escape observation. This freedom from the disease may have been due to the unusually dry weather during the growing seasons in the sections where the tung-oil trees are grown. In the numerous collections made in the field at various times during the seasons of 1930 and 1931 no typical leaf lesions were found. Cultures were made from all suspicious spots in these collections, but the characteristic pathogene was not found. The distribution of the disease is not well known, nor is the amount of damage it is capable of causing. During periods of moist, hot weather, especially with wind and rain to distribute the bacteria, the disease may become serious. According to Boyd,³ infection occurs on trees of all ages but is more likely to be destructive to the 1-year-old and 2-year-old stock.

In March, 1931, the senior writer examined several orchards in the vicinity of Gainesville, Fla., but found no evidence of the disease on either the new leaves or the dry, fallen leaves of the previous year's growth.

THE DISEASE

The following description of the disease is based on naturally infected leaves collected in Georgia and on artificially infected greenhouse plants in Washington, D. C.

The general appearance of infected leaves is shown in Figures 1 and 2. Mature, isolated lesions are distinctly angular in shape and are dark brown to black ("bister," "sepia" to "mummy brown")⁴ on the upper surface and somewhat lighter in color on the lower surface. A definite narrow border darker than the center is usually noted, and a wide, indefinitely margined, yellowish halo surrounds

¹ Received for publication Feb. 17, 1932; issued October, 1932. The investigation herein reported was originally planned by the senior writer in collaboration with O. C. Boyd, a collaborator of the Department of Agriculture, formerly at Thomasville, Ga., now extension plant pathologist of the Massachusetts State College. In accordance with a suggestion made by Doctor Boyd when he left Georgia, the work was carried out in collaboration with the junior writer, who was stationed at Thomasville.

² BOYD, O. C. A BACTERIAL DISEASE OF TUNG-OIL TREE. (Pathological Note) Phytopathology 20: 756-758, illus. 1930.

³ BOYD, O. C. Op. cit.

⁴ The color readings in this paper are based on the following publication: RIDGWAY, R. COLOR STANDARDS AND COLOR NOMENCLATURE. 43 p., illus. Washington, D. C. 1912.

very active infections. Under average conditions the progress of the infection is soon checked by the larger leaf veins, resulting in the formation of isolated angular lesions, 1 to 20 mm wide. The infected tissues are dull, dry, and opaque, often shriveling and cracking; there is no exudate. Infections are first evident as small, greenish, translucent spots, which soon become brown, opaque, and angular. The active advance of the infection is marked by a translucent border, which is lost when a vein becomes the boundary line. Only

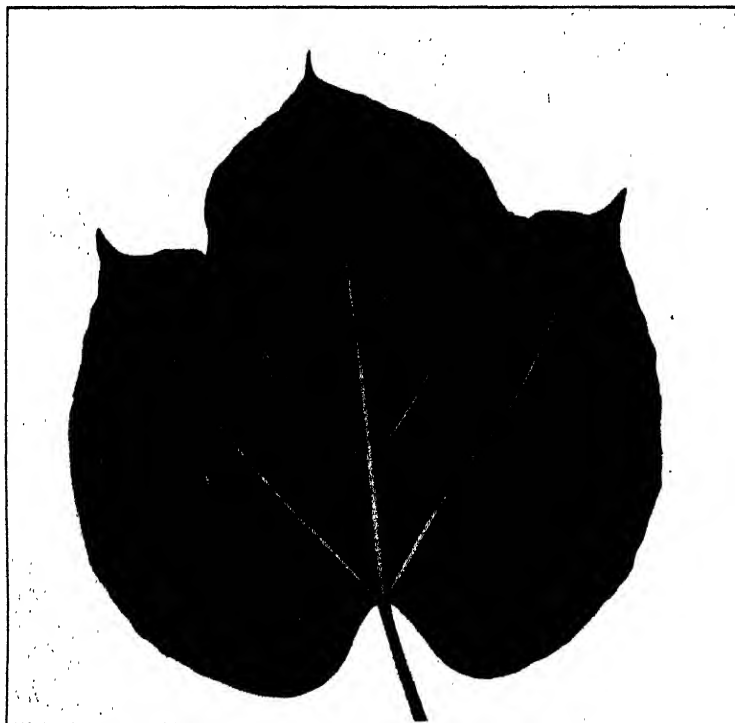


FIGURE 1.—Typical lesions on leaf of tung-oil tree, artificially infected. $\times \frac{3}{4}$

occasional mature lesions have translucent margins. When conditions are unusually favorable for the parasite, the infection spreads rapidly over large areas regardless of veins. (Fig. 3.) Lesions occur on all parts of the leaf blade; they are usually well distributed but are most numerous in areas where moisture is most abundant. The vascular system is not invaded, but veins within a lesion are dry and distorted.

Infections, which enter through the stomata,⁵ are often extensive enough to cause the destruction of the entire leaf.

⁵ Stomata are fairly numerous (35 to 40 to the square millimeter) on the lower leaf surface of *Aleurites fordii*. On the upper surface there are from 0 to 7 stomata to a square millimeter, and these few are located along the larger veins.

ISOLATIONS AND INOCULATIONS

The bacteria are abundant in the lesions and are easily isolated by the usual methods. Several isolations were made from the naturally infected leaves of the two collections of 1929, and numerous isolations have been made from leaves artificially infected. Dead, fallen leaves bearing numerous typical lesions were collected from a Georgia orchard in February, 1930. These leaves had probably been on the ground since October. Typical infectious bacteria were isolated from



FIGURE 2.—Leaf of tung-oil tree, four days after inoculation. Plants held at 28° to 32° C. and sprayed with water twice each day. $\times \frac{1}{2}$

these old leaves as late as April, 1930. Isolation tests in May, 1930, from these old leaves were not successful.

Small tung-oil trees were secured from Florida and established in a greenhouse in Washington, D. C.; a number of others were grown from seed. All the plants grew rapidly and, except when inoculated, remained free from disease.

The first inoculations were made in May, 1930, on 2-year-old trees, with subcultures of the bacteria isolated in November, 1929, from the leaves from Georgia. Bacteria from agar-slant cultures were suspended in water and sprayed on the plants, which were then placed in a moist inoculation chamber for 24 hours. Infection was evident in 3 to 4 days, and characteristic spots of good size developed in 6 to 8

days at a temperature of 28° to 32° C. From these induced lesions pure-culture reisolations, made May 14, 1930, were used on May 19, 1930, to inoculate tung-oil leaves, which were successfully infected. Numerous other inoculations in 1930 and 1931 have consistently produced the characteristic infection.

Infection was most active when the plants were kept in a moist atmosphere for 24 to 48 hours. A moderate amount of moisture

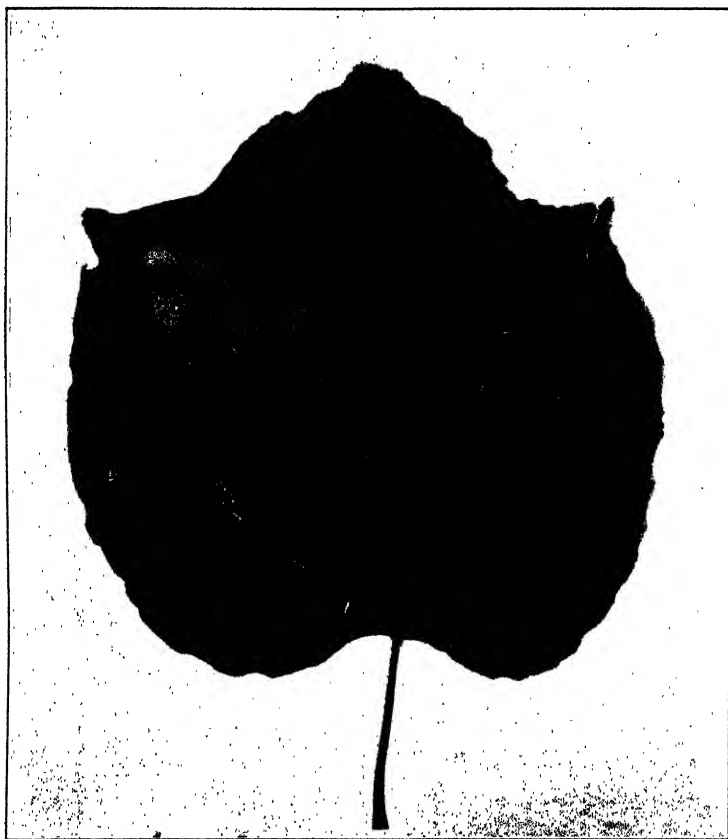


FIGURE 3.—Leaf of tung-oil tree, showing large areas infected under unusually favorable conditions of moisture. $\times \frac{1}{4}$

seems necessary for the production of large lesions. If the moisture is excessive, so that a film of water is retained on the leaves for hours, the infection is more intensive and, instead of isolated angular lesions, produces wide continuous areas of browned tissue, sometimes involving the whole leaf. Plants on the bench, under only the usual greenhouse conditions of moisture, were slow in showing infection, and the lesions were usually small.

Successful infections were secured by the junior writer in the field from inoculations made with two different strains of the bacteria.

One of these strains was isolated in March, 1930, from old weather-beaten tung-oil leaves from a Georgia orchard; the other was a reisolation from bean leaves infected with the tung-oil bacteria. These field inoculations were made in Georgia in 1931 during a period of dry weather, and the resulting lesions were small and rather slow in developing.

No infections have been secured on petioles or on stems of the inoculated tung-oil plants, either by means of needle-prick wounds or bruising, or by keeping the inoculated areas moist for several days.

The castor-bean (*Ricinus communis* L.), a plant related to the tung-oil tree, on which a bacterial leaf spot has been reported,⁶ was inoculated with the tung-oil organism under the same conditions of temperature and moisture as were the tung-oil plants. Infection occurred as leaf spots and occasionally as an interior rot of the hollow petioles. The infection was slow in developing, and leaf spots were never numerous. Leaf spots on *Ricinus* are pale yellow or light brown, irregularly circular, and mostly small, though a few spots reach 10 mm in diameter. The tissue lining the hollow stalk was blackened, while the exterior remained normal in appearance. Isolations from leaf and petiole infections of *Ricinus* were secured, and these when tested on tung-oil leaves produced the original type of infection. The characters of the bacterium previously reported⁷ as causing a leaf spot on *Ricinus* are quite unlike those of the bacterium described in the present study.

The bacteria causing the lesions on the tung-oil leaves have characters very similar to those of some known pathogenes of beans. It was at first thought that the disease had spread from some legume used as a cover crop in the orchards. However, cultural studies and comparisons showed that the tung-oil organism differed from all those described from the legumes. It is possible that the infection spread from a bacterial disease of some wild or cultivated plant growing in the vicinity of the tung-oil orchards.

Five varieties of beans (Red Kidney, Bountiful, Giant Stringless Green Pod, Black Valentine, and Refugee) are very susceptible to the tung-oil bacteria. When sprayed with the bacterial suspension, leaves of these varieties quickly developed isolated spots and large areas of thin, transparent tissue. Veins in the infected parts or adjoining them became bright reddish brown, the discoloration often extending a considerable distance beyond the leaf lesion and even into the petiole. The leaves were usually so seriously damaged that they fell in a few days. The slight vascular invasion in the leaf tissue did not extend into the stem; and, except in cases of direct contact, the infection did not spread to leaves other than those inoculated. When tested by inoculation on the tung-oil leaves, reisolations from all these bean infections proved that the infections were due to the tung-oil organism.

Tobacco plants were inoculated several times, but no infections resulted.

⁶ YOSHI, H., and TAKIMOTO, S. [BACTERIAL LEAF BLIGHT OF CASTOR BEAN.] Byôchû-Gai Zasshi (Jour. Plant Protect.) [Tokyo] 15:12-18. 1928. [In Japanese.]

⁷ YOSHI, H., and TAKIMOTO, S. Op. cit.

THE PATHOGENE

MORPHOLOGY

When grown on beef-infusion media 24 to 48 hours at 26° to 28° C., the causal organism is a rod with rounded ends, 1.1 to 3 μ long by 0.6 to 0.7 μ wide, its usual size being 1.8 by 0.7 μ . It occurs singly, in pairs, or in short chains. It is motile by means of 1 to 5 polar or, rarely, bipolar flagella. Capsules are present. No spores have been found. The organism is Gram-negative; not acid-fast. It stains readily with the usual bacterial stains.

CULTURAL CHARACTERS

BEEF-AGAR PLATES.⁴—On beef agar, under favorable conditions, colonies 1 mm in diameter appear in 24 hours. In two days well-isolated colonies are 1 to 4 mm wide, increasing in six days to 7 to 8 mm wide. At first white, or rather like boiled starch, and translucent, they soon become greenish white and transparent. They are smooth, shining, flat, mostly circular with an entire margin, occasionally becoming crenate to lobed. The interior is definitely striated or cross-hatched except in small, much-crowded colonies. Frequently a zone of pure white occurs at, or just within, the margin. Large colonies occasionally show 1 to 3 widely separated concentric markings. Bacteria isolated from young vigorous lesions often produce large, lobed colonies; otherwise the colonies from old lesions (2 to 7 months old) and from young lesions are alike. Submerged colonies are opaque, white, oval to spindle shaped. The medium is greened and has an unusual, rather sweetish odor.

BEEF-AGAR SLANTS.—Growth is thin, white, transparent, sometimes slightly viscid. The agar greens promptly. Numerous tiny crystals form on the slanted surface, and long, slender, needle-shaped crystals extend into the agar at right angles to the slant.

BEEF-AGAR STABS.—There is a moderate surface growth, greenish white. Growth occurs only in the upper few millimeters of the stab. In 6-months-old cultures numerous, short, rodlike crystals are present, uniformly distributed throughout the agar.

BEEF-BROTH.—A heavy, white, surface growth forms within 24 hours. This growth, at first tender and easily dissolved, is renewed after each disturbance, increasing in density until, after four to five days, it is more like a true pellicle. In undisturbed cultures, clouding below the surface is slow in developing. Narrow white rims are sometimes present. The sediment is whitish, translucent, rising in a spiral when rotated and dissolving easily. Greening of the broth begins at the surface and is complete in four to six days. Cultures four to eight weeks old have uniform clouding and no pellicle or rim. Clouding persists for four to five months.

POTATO CYLINDERS.—A rather scanty growth, smooth, dull yellowish white, develops on potato. The potato and the water are slightly browned.

THAXTER'S POTATO-DEXTROSE AGAR.—On this medium the growth is heavier than on beef agar and almost brown in the thicker parts.

CORN-MEAL AGAR.—No growth occurs on this medium.

COHN'S SOLUTION.—Growth in Cohn's solution with a pH of 5.2 is prompt and as good or better than in beef broth. There is a heavy surface growth, with numerous tiny floating crystals. Undisturbed cultures show horizontal bands of clouding, the heaviest at the top. The clouding and the production of surface crystals persist for weeks. There is a moderate, white sediment over a layer of tiny crystals. The medium becomes slightly green or pale straw color.

USCHINSKY'S SOLUTION.—There is a fairly heavy surface growth in 24 hours, and heavy, tender white pellicles in two days. Clouding becomes uniform by the tenth day. A fine blue-green fluorescence develops.

⁴ Unless otherwise noted, the beef media used were made with beef infusion and peptone and had a pH value of 6.8 to 7.0. Cultures were grown at room temperature.

PHYSIOLOGIC CHARACTERS

LIQUEFACTION OF BEEF GELATIN.—Well-isolated colonies in poured plates are 3 to 8 mm wide in six to seven days. They have a rather thick, slightly elevated, central area of granular to mottled structure, surrounded by a wide, very thin border, irregularly and often deeply lobed. Colonies are greenish white and transparent. In stab cultures there is good surface growth. There was no trace of liquefaction in plate or tube cultures held for eight weeks at 18° to 20° C.

LIQUEFACTION OF BLOOD SERUM.—Very scanty growth develops, and there is no clearing and no liquefaction.

PRODUCTION OF AMMONIA.—Ammonia production is very slight in a peptone-glucose dipotassium phosphate medium, and apparently absent in other media.

PRODUCTION OF HYDROGEN SULPHIDE.—Lead acetate paper suspended over cultures of various media indicates a very slight production of hydrogen sulphide.

PRODUCTION OF INDOL.—Cultures eight days old in 2 per cent peptone solution give a positive reaction for indol about one-half as strong as the check cultures of *Bacillus coli communis*.

TOLERATION OF SODIUM CHLORIDE.—In beef broth containing 1 to 2 per cent sodium chloride the bacteria grow readily. With 3 per cent sodium chloride the growth is considerably retarded, and very slight growth occurs in a 5 per cent solution.

HYDROLYSIS OF STARCH.—On starch-agar plates streaked with the organism, an area 7 to 10 mm wide beyond the growth is cleared within 10 days. In potato cylinders there is very little evidence of starch destruction.

RELATION TO FREE OXYGEN.—In solid media the growth occurs only at or very near the surface. There is no growth in the closed ends of fermentation tubes or on inoculated surfaces so covered as to exclude air.

REACTION IN MILK.—There is no separation of the milk, but old cultures (five to eight weeks old) are very slightly less opaque than the controls. There is a slight color change to cream-buff, or even a lighter color, and evaporation rims of chamois. Oil globules are deep rose pink. Litmus milk is blued. The endive blue to Dutch blue in 3-day-old cultures gradually deepens to dark green blue gray and then to slate gray in cultures three to six months old. There is a slight, fugitive reduction of the litmus in the lower third of the tubes. In one lot of medium containing less than the usual amount of litmus there occurred a practically complete reduction, lasting for 24 to 48 hours. There is a small amount of white sediment. Oil globules are deep rose to red.

METHYLENE BLUE IN MILK.—In two days the lower half of the tubes shows reduction and in five days the reduction is complete. The color returns within three weeks. In some tests there was a second reduction, followed by another return of color slightly greener than the controls.

REDUCTION OF NITRATE.—A decided nitrate reduction is evident in cultures 9 days old and a stronger reaction in cultures 12 to 18 days old, when the α -naphthylamine sulphanilic acid test is used. Parallel cultures tested with starch potassium iodide, and sulphuric acid show no nitrite reaction.

FERMENTATION OF CARBOHYDRATES.—Various sugars and alcohols were tested for fermentation. The carbohydrates were added to a synthetic agar made according to the formula in the Manual of Methods⁹ with brom cresol purple for the indicator. Growth and acid reactions were prompt from dextrose, galactose, glycerin, and mannite, the yellow color extending to the base of the slants in 24 hours. No growth and no color change occurred in saccharose, lactose, or maltose. Results were identical in five different tests.

With the same series of carbohydrates in fermentation tubes¹⁰ (same medium base but lacking agar) acid was evident in 24 hours from dextrose, galactose, and mannite. Glycerin showed acid reaction in two days. A few saccharose cultures showed slight growth and yellow color on the sixth day and others on the tenth day. After 15 days, growth and yellow color were observed in some of the maltose and lactose cultures. Cultural tests proved that these were pure cultures of the tung-oil organism. No gas was formed from any of these carbohydrates in the fermentation-tube cultures, and clouding was confined to the open ends of the tubes.

TOLERATION OF ACID AND ALKALI.—In beef broths ranging from pH 5.2 to 9.2 clouding occurs within 24 hours at pH 5.6 to 8.8, but is heaviest at pH 6.2 to 6.8. In two days there is slight growth at pH 8.9 and in five days at pH 5.4. In

⁹ SOCIETY OF AMERICAN BACTERIOLOGISTS, COMMITTEE ON BACTERIOLOGICAL TECHNIQUE. MANUAL OF METHODS FOR PURE CULTURE STUDY OF BACTERIA. 48 p., illus. Geneva, N. Y. 1928.

¹⁰ The fermentation tubes were steamed on three successive days. The agar tubes were sterilized under pressure.

Cohn's solution with a pH of 5.2 the organism makes a prompt, almost heavy growth.

RESISTANCE TO DESICCATION.—Bacteria from young beef-broth cultures were dried at room temperature on sterile cover glasses. At least a few bacteria survived for five weeks, but after drying for two days the majority of the covers failed to produce growth. The bacteria were alive in lesions on tung-oil leaves kept dry at room temperatures for four months. Bacteria in leaves lying on the ground in the orchard from the time of defoliation, October (?), until February of the following year retained vitality and pathogenicity until April.

VITALITY IN CULTURE MEDIA.—In beef media and in Cohn's solution the bacteria remain alive at least 11 months.

TEMPERATURE RELATIONS.—The best growth takes place between 27° and 28° C. At 10° to 12° beef broth clouded well in 48 hours. At 37° a very scanty growth, in the form of small isolated colonies on beef agar, appeared after two weeks. No growth was evident in liquid media at 37°. At 37.5 there was no sign of growth, but the organism remained alive for at least 14 days. At 35° moderate growth occurred in 24 hours and increased until by the sixth day the clouding was heavy. These cultures remained alive at 35° for at least 48 days. The thermal death point is 52°. Here again there is an uneven resistance. Some cultures held at 48°, 49°, 50°, and 51° for 10 minutes failed to grow, and occasionally those held at 52° clouded. There was never any growth in the tests at 53°.

TECHNICAL DESCRIPTION

Bacterium aleuritidis, n. sp.

A motile rod 1.1 to 3 μ long by 0.6 to 0.7 μ wide; 1 to 5 polar flagella, rarely bipolar; capsules present; no spores; Gram-negative; not acid-fast; colonies on beef agar are circular to slightly lobed, white, translucent, becoming greenish white and transparent. Clouds beef bouillon and forms a pellicle; gelatin not liquefied; produces a green fluorescence in all beef media; blood serum not cleared or liquefied; moderate diastatic action; acid without gas readily produced from dextrose, galactose, glycerin, and mannite; growth and production of acid slow from saccharose, lactose, and maltose; reduces nitrates; produces indol, ammonia, and hydrogen sulphide in slight amounts; aerobic; trace of peptonization of milk; litmus in milk shows a slight, fugitive reduction; litmus blues and remains blue. Excellent growth in Cohn's solution. Grows best at pH 6.2 to 6.8; irregular resistance to heat and desiccation; a few organisms survive cover-glass drying for five weeks; in herbarium leaf specimens and in dead leaves in the orchards the bacteria survive for at least four months. The optimum temperature for growth is 27° to 28° C., maximum temperature 87°. Dark brown, angular spots are produced on the leaves of the tung-oil tree (*Aleurites fordii*). Moderate infection is produced on the leaves of castor-bean (*Ricinus communis*), and serious infection on at least five varieties of beans (*Phaseolus* sp.).

Specimens of tung-oil leaves with lesions produced by this organism have been deposited in the pathological and mycological collections of the Bureau of Plant Industry, United States Department of Agriculture.

SUMMARY

A bacterial disease of the tung-oil tree (*Aleurites fordii* Hemsl.), as yet reported only from Georgia, produces brown, angular spots on the leaves. Defoliation results when considerable leaf tissue is affected. Moisture and heat favor the occurrence and spread of the disease. The bacteria probably remain alive and infectious in the lesions of fallen leaves in the orchards until new leaves appear the following spring. Such leaves are doubtless a factor in the recurrence of the disease. The causal bacteria have been isolated and used to produce typical lesions on tung-oil leaves. The bacteria are only slightly infectious on leaves of castor bean (*Ricinus communis* L.). On several varieties of bean (*Phaseolus* sp.) the infections due to these bacteria were very severe.

A description is given of the morphologic, cultural, and physiologic characters of the pathogene, for which the name *Bacterium aleuritidis* is proposed.

DETECTING PINK BOLLWORMS IN COTTONSEEDS BY THE X RAY¹

By F. A. FENTON, *Senior Entomologist, Division of Cotton Insects, Bureau of Entomology, United States Department of Agriculture*, and WILLIS W. WAITE, *Director, Southwestern Biological Laboratories*, in cooperation with the *Texas Agricultural Experiment Station*

In studying the hibernation of the pink bollworm, *Pectinophora gossypiella* Saund., and in comparing the effects of different combinations of plowing and irrigation as control measures for this insect, it has been necessary to examine hundreds of dry cotton bolls and cut thousands of seeds in order to determine mortality percentages. Owing to the extreme hardness of cottonseed this was extremely slow and tedious work. An effort was made, therefore, to discover a more rapid and efficient method of seed examination. Several samples were examined by means of a fluoroscope in a darkened room, but this procedure was not found effective. It was then suggested that an X-ray photograph might show the live worms inside of the seed. Although this operation would be too expensive for regular use with the limited number of seeds to be examined, it was of interest to determine the possibilities of the method. Several exposures were made of both delinted and undelinted ginned seed, as well as of locks of cotton with the seeds in their normal position. It was found possible to detect immediately the presence of live or dead larvae within the seed. The results were so striking that the method seems well worth recording.

As previously stated, delinted seed, seed direct from the gin, or seed with the cotton attached can be used. Ginned seed is just as good as delinted seed and eliminates the work of delinting. Unginned seed occupies too much room on the film, and inasmuch as seeds in the cotton are often superimposed upon one another, it may be impossible to get a clear picture of all of them.

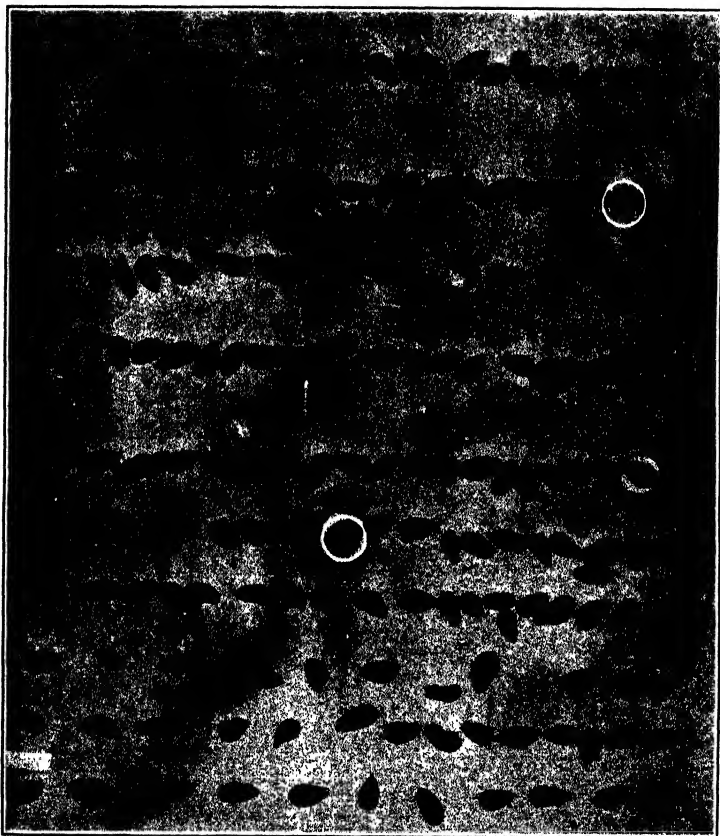
The seeds are best put out in rows, one layer deep, over the film. The rows may be close together and the seeds in the rows may touch one another. With this arrangement the film can be examined easily, and it is possible to count both the infested and the imperfect seeds.

If it is desirable to check up the bad seeds found in the film, the seeds should be placed on some sort of a channel or cover over the film so that the film can be removed without disturbing the seeds. A film mounted in a cardboard holder is sufficient. Intensifying screens are unnecessary. Very little exposure is needed, so any sort of X-ray machine is suitable for making the pictures. With a little experience just the right exposure can be determined.

¹ Received for publication Feb. 1, 1932; issued October, 1932.

The examination of the film is aided by the use of a good hand lens. The perfect seeds show up well, without defects. The imperfect seeds and infested seeds are not so easily distinguished, since many imperfect seeds are in this condition because the worms have bored into them. In other seeds the worms are dead and shriveled. The live worms give a fairly characteristic picture, and after a little study and checking of the film with actual examination of the seed, one soon becomes familiar with their appearance (pl. 1) so that it is sufficient to rely on the picture alone for their detection.

It is possible to photograph a thousand seeds at once, and it takes only a few minutes to arrange the seeds and make the exposure. By having plenty of films ready a large number of photographs can be taken in a short time. These films can then be developed and kept as a permanent record.



X-ray photograph of cottonseeds, showing two (in white circles) infested with live pink bollworms and two (in black) infested with dead worms

THE EFFECT OF THE DEGREE OF SLOPE ON RUN-OFF AND SOIL EROSION¹

By F. L. DULEY, *Professor of Soils*, and O. E. HAYS, *Graduate Assistant in Soils, Department of Agronomy, Kansas Agricultural Experiment Station*

INTRODUCTION

The degree of slope of land has long been considered one of the major factors governing the amount of run-off and soil erosion. Few attempts, however, have been made to establish even the most simple mathematical relationships between the degree of slope of land and the amount of run-off and erosion. While at the university of Missouri, the senior author, in collaboration with Prof. M. F. Miller,² reported the results of erosion measurements begun in 1917 on a 3.68 per cent slope. In 1924 additional plots were installed on a 6 per cent and on an 8.25 per cent slope.

The work on these plots, involving three slopes, has been continued at the Missouri station, and a preliminary report has been made by Miller.³ The chief difficulty encountered in this type of study is that when slopes of different degrees and in somewhat separated locations are chosen, the soil profiles are not identical. The surface soils also are likely to be widely different in absorbing power since they have developed under a different set of conditions. In the work reported in the present paper two methods have been used for determining the effect of slope on the amount of run-off and erosion, in which attempts have been made to eliminate the soil variations referred to above.

METHODS

LABORATORY TESTS

One of the two methods may be termed a laboratory method. A galvanized-iron tank 24 inches wide, 28 inches deep, and 10 feet long was surrounded by heavy framework, as shown in Figure 1. This method was somewhat similar to that reported by Lowdermilk⁴ for studying certain questions in connection with forest soils. A silty clay loam soil with a fairly heavy clay subsoil from the agronomy farm was removed in 6-inch layers from a plot exactly the same size as the tank. The soil was placed in the tank in the same order as it existed in the field and was carefully tamped in until it occupied the same volume as it did in the field. Before putting in the soil a 2-inch layer of sand was placed in the bottom of the tank to provide thorough underdrainage, and the excess water was removed through a small drainage tube. The sides of the tank extended 2 inches above the surface of the soil, except at one end where the iron was turned

¹ Received for publication Jan. 19, 1932, issued October, 1932. Contribution No. 214 Department of Agronomy, Kansas Agricultural Experiment Station.

² DULEY, F. L., and MILLER, M. F. EROSION AND SURFACE RUNOFF UNDER DIFFERENT SOIL CONDITIONS. Missouri Agr. Expt. Sta. Research Bul. 63, 50 p., illus. 1923.

³ MILLER, M. F. EROSION AS A FACTOR IN SOIL DETERMINATION. Science (n. s.) 73: [79]-83. 1931.

⁴ LOWDERMILK, W. O. INFLUENCE OF FOREST LITTER ON RUN-OFF, PERCOLATION, AND EROSION. Jour. Forestry 28: 474-491, illus. 1930.

down level with the soil to permit run-off. When complete this tank equipment with soil and water weighed about 2.5 tons.

Differences in slope of the soil surface in the tank were obtained by raising one end by means of jackscrews or a differential hoist. A slope of about 20 per cent was as much as it was found possible to give this

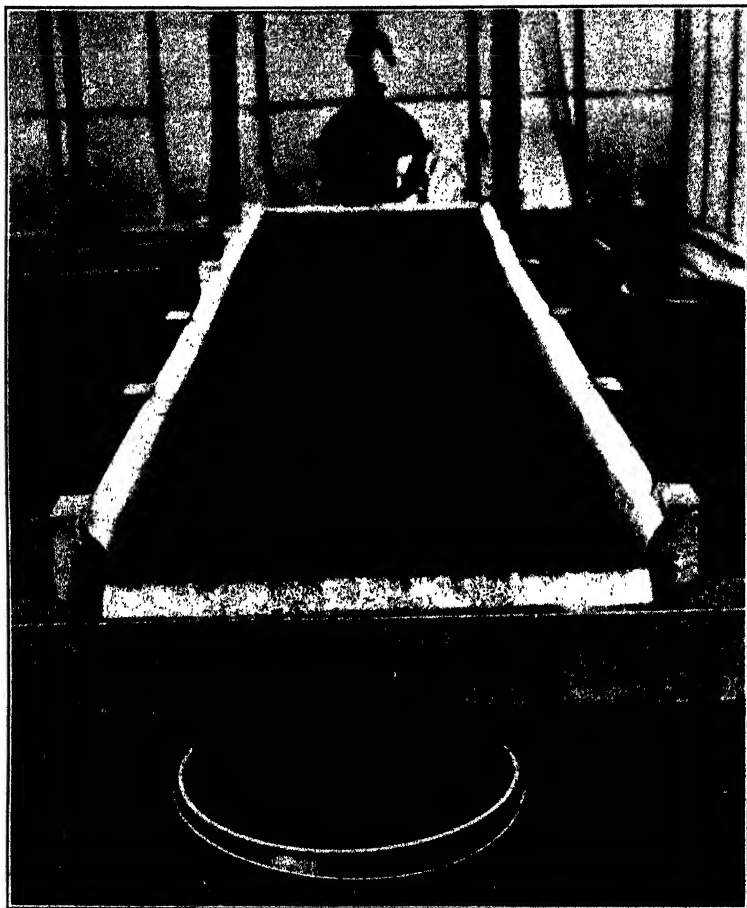


FIGURE 1.—Tank filled with soil and used in greenhouse to determine the effect of slope of the soil on run-off and soil erosion. By raising one end of the tank any desired degree of slope may be obtained.

block of soil without throwing undue strain on the tank and framework. A later test, however, was made with a 40 per cent slope.

FIELD TESTS

A second method of determining the effect of slope on run-off and erosion was carried out in the field. In this work an attempt was made to eliminate soil variation by using plots placed at different

angles on the same slope. By properly placing the plots, slopes varying from 0° to the maximum slope of the hillside can be obtained. This method is illustrated in Figure 2.

The plot was surrounded by strips of galvanized iron set in the ground to depths of about 8 inches. The iron extended about 2 inches above ground, and the upper edge was turned to prevent bending. At the lower end the edge was turned down about 2 inches until the top was on a level with the soil surface, and the narrow apron thus formed turned down into a small metal gutter which was used for carrying the run-off water and eroded soil into a bucket.

The plots were then leveled crosswise so that there was a slope in only one direction, and the slope lengthwise the plot was made uniform by carefully checking with a line. Some objection might be raised to this leveling process, but fairly deep soils were chosen so that the change in depth of surface soil would not be great and the



FIGURE 2.—Plots located at different angles on a hillside to obtain different degrees of slope on the same soil. The iron frame used to surround the plots has been removed

subsoil would not be disturbed. Since the plot used was only 34.85 inches wide, it was necessary to transfer only a small amount of soil from the upper to the lower side in order to make the surface level crosswise of the plot. The soil was then spaded to a uniform depth and carefully worked on the surface with a hoe and garden rake so that the amount of loose soil was the same over the entire plot. Care was taken that there were no holes or loose places which would cause irregular settling or uneven absorption of water.

Water was applied to the soil by means of a sprinkling can. The rate of application was carefully timed so that any desired rate of rainfall could be imitated. Several mechanical methods of applying water by means of sprinklers were tried, but it was found that, if carefully used, the sprinkling-can method was the most satisfactory.

The run-off water and eroded soil were collected and weighed, and samples were taken for determining the amount of eroded soil.

EXPERIMENTAL RESULTS

TANK EXPERIMENTS

EFFECT OF SLOPE ON RUN-OFF

The tank of soil was thoroughly soaked with water before any measurements were made. This was done so that the results from different slopes would be comparable. The water was then applied at the rate of 1 inch an hour. By carefully timing the application, it was possible to keep an almost constant application of water. The amounts of run-off and erosion were first determined for a zero slope, or level land. The slope was then increased and the water and soil loss determined. The results of four sets of determinations are shown in Table 1. The average of the four determinations have been plotted on a curve. (Fig. 3.) The most significant thing about these results,

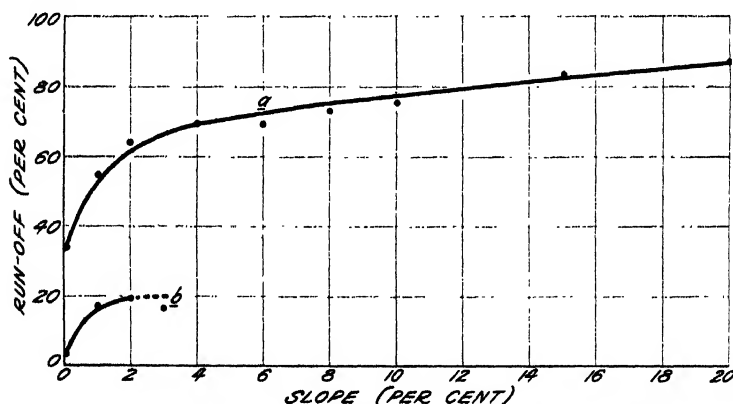


FIGURE 3.—Relation between the degree of soil slope and the percentage run-off: (a) Results obtained in Kansas when using soil tank in greenhouse with silty clay loam soil and (b) at Spur, Tex., with field plots. Broken part of this curve shows where it might be expected to fall for 3 per cent slope

as indicated by the curve, is the very rapid rise in the rate of run-off as the slope is increased from 0 to 2 per cent, followed by a more gradual rise to between 3 and 4 per cent. After this there is a very much slower rise in the curve. These results seem to indicate that on level land there may be a considerable amount of run-off, but when there is a slight slope the water is less hampered by the very slight depressions and runs off in much greater amounts before it can be absorbed; that is, it will not be held on the land much longer than the duration of the rain. With a still further increase in slope, the increase in run-off becomes relatively less because the water on any slope is running over the land for the entire duration of the rain and thus time is afforded for absorption. Any run-off that may be taking place at the end of the rain will cease within a short time whether the slope is slight or steep.

TABLE 1.—Effect of slope of tank on run-off water from surface of soil

[Water applied at rate of 1 inch an hour; size of tank 2 by 10 feet]

Slope	Quantity of run-off water					Average run-off
	Jan. 25, 1931	Jan. 31, 1931	Feb. 21, 1931	Feb. 28, 1931	Average	
Per cent	Pounds	Pounds	Pounds	Pounds	Pounds	Per cent
0	38.1	—	20.4	47.2	35.2	33.85
1	57.8	52.2	45.8	70.5	56.5	54.33
2	61.8	65.9	59.5	76.8	66.0	63.47
4	72.0	70.5	62.8	81.2	71.6	68.86
6	80.8	62.3	72.7	—	71.9	69.15
8	83.3	70.7	74.7	—	76.2	73.28
10	82.3	72.6	76.3	81.5	78.2	75.21
15	—	—	—	86.8	86.8	83.48
20	—	—	—	89.9	89.9	86.45

• 4.16 per cent slope.

EFFECT OF SLOPE ON SOIL EROSION

An examination of Table 2 and Figure 4 will show that the effect of slope on erosion is almost opposite to that on run-off. The amount

of soil removed increased very slowly as the slope was increased up to about 3 or 4 per cent. Then with increasing slope there was a very rapid rise in the amount of soil removed. This shows that with only a very low gradient when the water runs at a relatively slow rate, the soil is not picked up, and the water flows away without carrying much soil, except the lighter particles; but when the slope is increased, the water runs faster, and even though the average depth may be less, a far greater amount of soil is removed. The number of pounds of water required to remove 1 pound of soil decreases rapidly with increasing slope, as shown by Table 2. In a few cases the 0 and 1 per cent grades required a smaller amount of water to remove a pound of soil than did the next higher grades. Further tests are needed to establish this point definitely.

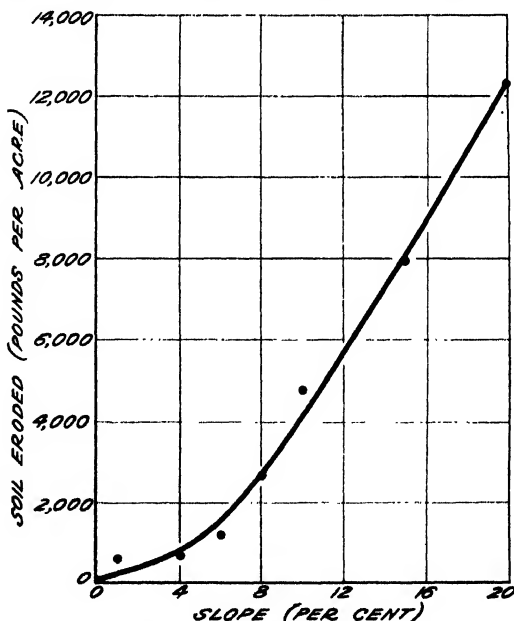


FIGURE 4.—Relation between the degree of soil slope and the quantity of soil eroded from silty clay loam soil in the tank in the greenhouse. Dots show average actual values

In the fall of 1931 the tank was filled with sandy loam with a sandy subsoil from the soil near St. George, Kans., where the field tests were made. The soil was thoroughly soaked with water so that drainage was taking place from the bottom before the readings were started. This made it possible to have the soil in approximately a uniform condition during the time the readings were being made for the various slopes. On November 2 water was applied equivalent to 1 inch an hour. One week later another set of readings was made. At this time the water was applied at the rate of 1 inch in 30 minutes. Tables 3 and 4 give the results obtained.

TABLE 2.—*Soil eroded (pounds) from silty clay loam in greenhouse tank at different percentages of slope*
[Water applied at rate of 1 inch an hour]

Slope	Soil eroded						Run-off required to remove 1 pound of soil
	Jan. 24, 1931	Jan. 31, 1931	Feb. 21, 1931	Feb. 28, 1931	Average	Per acre	
Per cent	Pounds	Pounds	Pounds	Pounds	Pounds	Pounds	Pounds
0	0.037	—	0.067	0.038	0.054	117.6	651
1	.090	0.429	.287	.265	.267	581.5	211
2	.103	.275	.185	.160	.180	392.0	367
4	.238	.166	.171	.559	.283	616.4	253
6	.587	.260	.722	—	.523	1,139.1	137
8	1.269	1.002	1.259	—	1.176	2,561.3	65
10	1.289	3.064	1.751	2.502	2.151	4,084.6	36
15	—	—	—	3.591	3.591	7,821.2	24
20	—	—	—	5.563	5.566	12,122.7	10

* 4.16 per cent slope.

TABLE 3.—*Soil eroded and run-off from sandy loam in greenhouse tank at different percentages of slope, November 2, 1931*
[Water applied=1 inch in one hour]

Slope	Soil eroded—		Run-off water		Run-off required to remove 1 pound of soil
	Per plot	Per acre			
<i>Per cent</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Per cent</i>	<i>Pounds</i>
0.5	0.0897	195.4	71.09	68.36	792.5
2.0	.0519	113.1	73.62	70.80	1,417.4
4.0	.0880	191.6	78.74	75.73	895.1
8.0	.4559	993.0	81.53	78.41	178.8
16.0	11.559	25,175.5	81.74	78.61	7.07

TABLE 4.—*Soil eroded and run-off from sandy loam in greenhouse tank at different percentages of slope, November 7, 1931*
[Water applied=1 inch in 30 minutes]

Slope	Soil eroded—		Run-off water		Run-off required to remove 1 pound of soil
	Per plot	Per acre			
<i>Per cent</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Pounds</i>	<i>Per cent</i>	<i>Pounds</i>
0.5	0.1643	357.8	86.11	82.81	524.1
2.0	.2138	465.7	93.08	89.52	435.3
4.0	.3551	773.4	93.78	90.19	264.1
8.0	2.573	5,604.0	95.59	91.93	37.2
16.0	26.44	57,586.3	96.54	91.88	3.6

It may be seen from the results that the loss of soil was very low up to a 4 per cent slope. Even with the 8 per cent slope only a relatively small amount was lost when the water was applied at the rate of 1 inch an hour. When the slope was doubled to 16 per cent, the erosion was increased to 25.3 times the amount eroded at 8 per cent. With the heavier applications of water the erosion on the 8 per cent slope was increased to 5.6 times the amount of erosion from the lighter application. When the slope was increased to 16 per cent and water applied at the rate of 1 inch in 30 minutes, the erosion was increased to 10.28 times that on the 8 per cent slope. With an application of 1 inch of water an hour on 16 per cent grade the loss was equivalent to 12.5 tons an acre, and with an application of 1 inch in 30 minutes the loss of soil was equivalent to 28.8 tons an acre.

The run-off from this soil was high and did not show as much variation in amount as in previous tests, but did show the same general type of curve as has been found in both tank and field tests.

FIELD DETERMINATIONS

RUN-OFF FROM SILTY CLAY LOAM

The field determinations were obtained from a plot 34.85 inches wide and 25 feet long or one six-hundredth acre. The soil used was a heavy silty clay loam with a silty clay subsurface. This grades into a heavy clay at 12 to 14 inches. The slope was varied from 0.96 to 5.96 per cent, which was as steep as could be obtained on this land. (Table 5.) The results show the same trend as did the results from the tank, but there was possible a variation of only about 6 per cent in the slope. It may be seen here as in the results from the tank that there is a very great increase in the run-off up to about 2 to 3 per cent. After this, the increase in run-off with increase in slope is much slower.

TABLE 5.—Soil eroded from a 1/600-acre plot of silty clay loam on the agronomy farm as related to rate of water application, percentage of slope, and run-off, 1931

Date	Rate of water application *	Slope	Run-off water		Soil eroded	Soil eroded per acre	Run-off required to remove 1 pound of soil
		Per cent	Pounds	Per cent	Pounds	Pounds	Pounds
June 18	1 inch in 30 minutes.....	0.96	1.986	0.53	0.0142	8.51	139
	1 inch in 1 hour.....		14.875	3.94	.0246	14.75	604
	(1) 1 inch in 30 minutes.....		174.111	46.14	.4889	293.11	356
	(2) 1 inch in 30 minutes.....		189.656	50.26	.4445	266.5	426
	1 inch in 30 minutes.....		35.045	9.29	.1544	92.57	226
	1 inch in 1 hour.....	2.12	86.900	23.03	.2997	179.69	289
	(1) 1 inch in 30 minutes.....		238.966	63.32	.6337	379.93	377
	(2) 1 inch in 30 minutes.....		237.697	62.99	.5026	301.33	472
	1 inch in 30 minutes.....		69.564	18.43	.5352	320.94	129
	1 inch in 1 hour.....		120.852	32.03	.4472	268.12	270
June 17	(1) 1 inch in 30 minutes.....	3.8	248.646	65.89	.9540	571.97	260
	(2) 1 inch in 30 minutes.....		267.006	70.75	.7940	476.04	336
	1 inch in 30 minutes.....	5.96	77.616	20.57	.4838	290.06	160
	1 inch in 1 hour.....		128.794	34.13	.7054	422.92	162
	(1) 1 inch in 30 minutes.....		249.152	66.02	1.2477	748.06	199
	(2) 1 inch in 30 minutes.....		237.735	68.30	1.3647	818.20	188

* Numbers in parentheses indicate different runs, No. 2 followed immediately after No. 1, which gave an application of 2 inches in 1 hour.

A comparison of the data for different rates of application will show that there is a much greater run-off when water is applied

equivalent to heavier rainfall. This difference is due mainly to the fact that the absorption of water during a water application equivalent to 1 inch an hour is approximately the same as at a 2-inch-an-hour rate.

EROSION ON SILTY CLAY LOAM

The amounts of soil lost from the plots used in these tests were in some instances not so consistent, especially with the lighter applications of water, as the results from a number of other determinations. There were a few cases in which the amount of eroded soil did not fall at the expected place on the curve. The reason for these slight irregularities seems to be due to the fact that it is usually more difficult to get consistent results with the lower slopes and lighter rainfall because slight variations in the surface have a relatively greater effect than on the steep slopes or with heavy rainfall. The general trend of the results with heavier water applications on this soil, however, were similar to those obtained for the same slopes in the other tests.

RUN-OFF FROM SANDY LOAM

A sandy soil with a sandy subsoil located north of St. George, Kans., 9 miles east of Manhattan, was used for additional tests. The maximum slope here was nearly 12 per cent. At the time these tests were made the soil was extremely dry and no run-off was obtained on the more gentle slopes until about 2 inches of water had been added. (Table 6.) These results followed very much the same trend as those obtained with the tank and with the soil on the agronomy farm. With a 2-inch application of water in 30 minutes after 3 inches had been applied, the increase in run-off was more rapid with the first increase in slope. The run-off from the steeper slopes in this case dropped below that of the intermediate slopes. (Table 6.) No satisfactory explanation can be given for this departure from the regular trend of this curve, but it was probably due to slight irregularities in this sandy soil on the 2 and 4 per cent slopes.

TABLE 6.—*Soil eroded from a 1/600-acre plot of sandy loam as related to rate of water application, percentage of slope, and run-off, St. George, Kans., July 16-17, 1931*

Rate of water application	Slope	Run-off water		Soil eroded	Soil eroded per acre	Run-off required to remove 1 pound of soil
	Per cent	Pounds (°)	Per cent (°)	Pounds (°)	Pounds (°)	Pounds
1 inch in 30 minutes.....	0.6	(°)	(°)	(°)	(°)	-----
1 inch in 1 hour.....		(°)	(°)	(°)	(°)	-----
1 inch in 30 minutes.....		0.799	0.21	0.0088	5.27	91
2 inches in 30 minutes.....	2.2	179.50	23.78	.2743	164.4	654
1 inch in 30 minutes.....		(°)	(°)	(°)	(°)	-----
1 inch in 1 hour.....		(°)	(°)	(°)	(°)	-----
1 inch in 30 minutes.....	4.08	52.83	14.00	.0746	44.76	708
2 inches in 30 minutes.....		438.05	58.04	.4547	272.8	963
1 inch in 30 minutes.....		(°)	(°)	(°)	(°)	-----
1 inch in 1 hour.....	7.72	(°)	(°)	(°)	(°)	-----
1 inch in 30 minutes.....		68.43	18.13	.2670	160.07	256
2 inches in 30 minutes.....		457.54	60.49	3.3664	2,018.3	136
1 inch in 30 minutes.....	11.36	1.18	.31	.0192	11.5	61
1 inch in 1 hour.....		2.27	.60	.0227	13.6	100
1 inch in 30 minutes.....		56.82	15.06	.5819	348.9	98
2 inches in 30 minutes.....	11.36	359.72	47.67	7.1769	4,302.9	80
1 inch in 30 minutes.....		2.90	.74	.2943	176.4	12
1 inch in 1 hour.....		8.12	2.15	.3702	221.9	20
1 inch in 30 minutes.....	11.36	77.68	20.59	3.9212	2,291.0	20
2 inches in 30 minutes.....		353.82	47.14	28.881	17,316.6	12

* None.

EROSION ON SANDY LOAM

The soil eroded from the plots when water was applied heavily is represented in the curve in Figure 5. The type of curve obtained on these field plots is very similar to that obtained with the tank and with the silt loam soil. There is a very slow and gradual rise in the amount of erosion up to about 3 to 4 per cent slope, when the curve rises rapidly and erosion becomes exceedingly severe with very rapid rise in the curve at about 7 per cent slope and greater. A 2-inch rain falling in a 30-minute period caused seven times as much erosion on this soil on the steeper slopes and about thirty times as much on the gentle slopes as a 1-inch rain falling in the same time. This difference is due partly to the fact that sand grains are difficult to move, but when sufficient water is running over the surface they are picked up readily and carried down the slope.

SOIL EROSIVENESS

By comparing the amount of erosion shown in Table 2 with that in Table 3 it will be seen that there are some striking differences in the rate of erosion for silty clay loam and sandy loam soils. For the lower slopes, 2 to 8 per cent, there is a much higher erosion from the silty clay loam soil. The 8 per cent slope on the silty clay loam soil gave 158 per cent increase over the amount eroded from the sandy soil, whereas the 16 per cent slope on the sandy soil gave an increase of 222 per cent over the amount lost from the 15 per cent slope on the silty clay loam soil. Thus the relative erosiveness of the two soils was completely reversed simply by changing the slope of the land.

These figures show the difficulty of classing some soils as erosive and others as nonerosive on the basis of certain physical constants as has been attempted by Middleton.⁵ It would appear from these

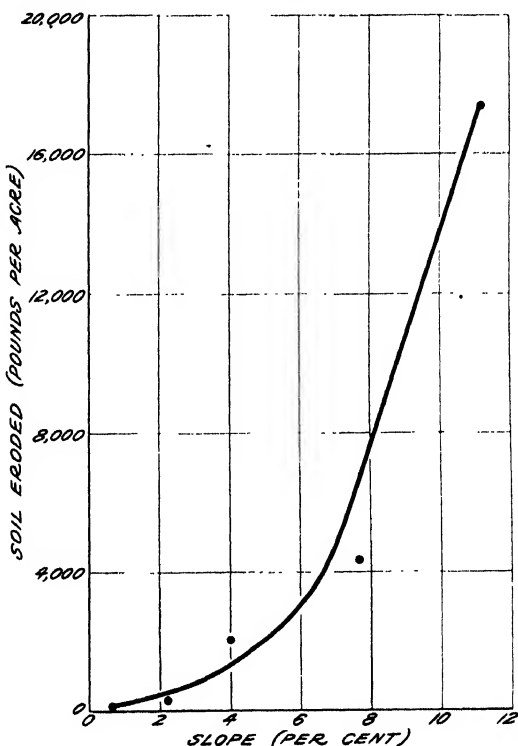


FIGURE 5.—Relation between the degree of slope and the quantity of soil eroded from a sandy loam soil to which water was applied at the rate of 2 inches in 30 minutes after 3 inches had been applied

⁵ MIDDLETON, H. E. PROPERTIES OF SOILS WHICH INFLUENCE SOIL EROSION. U. S. Dept. Agr. Tech. Bul. 178, 16 p. 1930.

data that erosiveness is not merely a specific property of the soil itself, but the conditions under which the erosion takes place must also be given consideration. Middleton found in the soils with which he worked that the nonerosive soils were low in sand and high in colloids. In the work herein reported, the relative erosiveness of a sandy soil has been found to depend largely on the degree of slope and the rate of rainfall.

In comparing the silty clay loam with the sandy soil it will be seen that with the lower slopes the light soil grains may be carried away from the silt loam, when there is not sufficient speed to the water to pick up the soil particles in the sandy soil. With increase in the slope to 16 per cent the water flows with enough force to carry the larger and heavier particles from the sandy soil; consequently the erosion

is greatly increased and far surpasses that from the silty clay loam soil.

The amount of water required to remove 1 pound of soil on the 8 per cent slope was 65 pounds for the silty clay loam and 179 for the sandy loam, but on the 15 per cent slope on the silty clay loam it required 24 pounds, and on the 16 per cent slope on the sandy soil only 7 pounds of run-off water were required to remove 1 pound of soil.

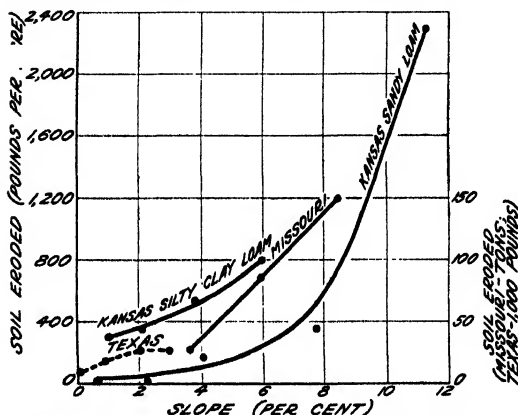


FIGURE 6.—Comparison between soil-erosion results obtained in Kansas with artificial watering and those obtained as annual averages at Columbia, Mo., and Spur, Tex., under natural conditions and on larger plots

It would therefore seem necessary to take into consideration the degree of slope, the rate of rainfall, and possibly other factors before the relative erosiveness of two soils can properly be expressed.

APPLICATION OF RESULTS

The question naturally arises as to whether the results obtained by these methods on small plots with water applied artificially, can be applied to field conditions. Some comparisons have been made with results from other stations. In Figure 6 will be found a curve showing results from the Missouri station at Columbia reported by Miller.⁶ These results give almost a straight-line curve, but this follows the general direction of the curve for the sandy loam soil used in these tests for the same degree of slope. When differences in location and methods are considered, this probably could be regarded as a reasonably good agreement. Since in the Missouri tests only three points on the curve are represented, it is impossible to show the trend for a wide range of slope since it has been found in the present work with small plots that the greatest increase in erosion is

⁶ MILLER, M. F. Op. cit.

to be found at about the point of the lowest slope used in the Missouri tests. A similar comparison was attempted with the results obtained by Conner, Dickson, and Scoates⁷ at the Spur, Tex., station. Their results were obtained on slopes ranging from 0 to 3 per cent. This was below the point of greatest increase found in the tests herein reported. The chief difference in the results is the fact that in Texas a large increase in erosion was obtained with the 1 per cent slope as compared with the zero slope. The increase from 1 to 3 per cent slope was much less marked. Another difference in methods is that the different slopes in Texas were produced by artificially adjusting some of the slopes for the entire length of the plot, whereas in the present work the natural slope was used for the length of the plot and the only adjustment of the surface was in leveling across the plot, which in most cases did not disturb much soil.

The amount of run-off obtained in the Texas experiments is represented by the curve *b* in Figure 3. This curve is very similar to that obtained with the tank, except that the 3 per cent slope has given less run-off than has the 2 per cent slope. Conner and his coworkers state that this is probably due to the building up of the soil to obtain the 3 per cent grade.

It would seem from these few comparisons at least, that the methods herein reported give results comparable with those obtained on larger plots and conducted under conditions of natural rainfall. They have the distinct advantage of affording opportunity for a large number of repetitions of comparable conditions as well as the possibility of studying a great number of different conditions bearing on a given problem, and in addition to this may be used for a wide variety of problems. Permanent plots placed at different angles on a hillside could be used to excellent advantage for determining the effect of slope under normal rainfall conditions extending over a long period.

SUMMARY

Determinations of run-off and erosion were made by means of water applied to soil artificially to simulate rainfall.

In one case a tank, which could be tilted so as to vary the degree of slope of the surface, was filled with soil and used to study the effect of slope on run-off and erosion.

In another test the plots were placed at different angles on a hillside so that the slope ranged from level to that of the steepest part of the hill. By properly locating the plots large variations in soil profile could be avoided.

The results from the two methods checked very well and indicate that the one to be used will depend on the type of problem to be studied.

The run-off was found to increase rapidly as the slope increased from zero to about 3 per cent grade. The increase in run-off was then very slight for each per cent of increase in slope.

The soil eroded increased very gradually until the slope was about 4 per cent, then the increase was found to be more rapid up to about 7 or 8 per cent, after which there was a still greater increase in the rate at which the soil was removed from the plots.

⁷ CONNER, A. B., DICKSON, R. E., and SCOATES, D. FACTORS INFLUENCING RUNOFF AND SOIL EROSION. Tex. Agr. Expt. Sta. Bul. 411, 50 p., illus. 1930.

The amount of run-off water required to erode 1 pound of soil decreased rapidly as the slope increased from 1 per cent up to about 10 per cent, after which the decrease was gradual and slight. In some cases the water required to erode 1 pound of soil was less for the 0 and 1 per cent slopes than for a 2 per cent slope.

Soil erosiveness is shown to depend not merely on the physical properties of the soil, but also on the degree of slope and possibly on several other factors. A silty clay loam gave greater erosion on the lower slopes whereas a sandy soil gave more erosion than did the silty clay loam on steep slopes.

The results obtained on large plots in Missouri and Texas have been shown to correspond reasonably well with the results obtained in these tests. This would tend to indicate that small plots having water applied artificially may be used for studying a large number of problems in connection with soil erosion.

DETERIORATION IN SHELLED GREEN PEAS HELD A FEW DAYS IN STORAGE PRIOR TO CANNING¹

By Z. I. KERTESZ, *Associate in Research*, and E. L. GREEN, *formerly Associate in Research, Department of Chemistry, New York State Agricultural Experiment Station*²

INTRODUCTION

It is the task of the canners to preserve the flavor and texture of fresh green peas as far as possible. The success or failure of their efforts is mostly decided before the operations within the cannery start, that is to say, by the quality of the harvested peas and by the events and the lapse of time between the cutting of the vines and the actual canning process. To postpone harvesting a given field for a day or so would damage the quality of the canned product, and every delay in the regular progress of the harvested peas to the cannery inevitably means some loss of flavor of the canned peas. Such delays lead to deplorable congestion at the cannery on certain days. It has occurred to a number of workers that peas might be held in cold storage, but the conditions to be met are still to be determined. It is believed that this can be done only after a better understanding of the biochemical reactions that are involved.

The results of a preliminary experiment on holding peas are presented in this paper. This trial was planned as a further step in the study of the reactions in cannery peas which were chosen as the first subject of biochemical investigations on vegetables started at this station in 1929.

The changes that occur in peas that have been removed from the pods are unexpectedly rapid and complex. In a previous study of these changes, the senior writer (5)³ found that an hour at room temperature (25° C.) produces a considerable alteration not only in flavor, but also in chemical composition.

The research department of the American Can Co. has made very extensive trials in holding shelled peas. The experiments showed that slow cooling did not check the objectionable changes before excessive damage had been done to the quality of the peas and that rapid cooling always imparted a sharp flavor. Trials by other investigators on rapid cooling, however, failed to confirm the occurrence of the sharp flavor.

¹ Received for publication Feb. 8, 1932; issued October, 1932.

² The authors wish to express their thanks to the American Can Co. for the privilege of reading the unpublished report of the experiments of the research department and for canning the experimental samples; to the Geneva Preserving Co. for its cooperation; to the Division of Fruits and Vegetables, Bureau of Agricultural Economics, U. S. Department of Agriculture; to the Wisconsin Department of Markets; to Strasburger and Siegel, consulting chemists, Baltimore; and to the members of the agricultural committee of the Association of New York State Canners (Inc.), for the scorings of the canned peas. They also wish to thank J. R. Magness, V. R. Boswell, J. W. Roberts, and C. A. Magoon for reading the manuscript.

³ Reference is made by number (italic) to Literature Cited, p. 370.

In an account of the work done by the research department of the National Canners' Association, Smith (7) reported that he was unable to find any sharp taste in pea samples cooled and held in cold storage. He states that the greatest change in flavor takes place in the first six hours of storage. Afterwards the loss of flavor in cold storage is very slow.

On account of these experiments by Smith it was believed that another experiment on rapid cooling was warranted. The object in making similar experiments was to study the changes in the chemical composition of the peas during storage and to compare these findings with the opinions of competent judges who scored the canned peas. It was of interest, in addition, to study the formation of the sharp taste, which certainly should be associated with detectable changes in the chemical composition of the peas.

EXPERIMENTAL METHODS

In this preliminary work three different treatments were used, viz, holding peas at room temperature without treatment (series O), cooling and storing the peas at 30° F. without blanching (series C), and cooling and storing at 30°, after blanching (series B).

The peas were collected on July 7, 1931, at Waterloo, N. Y. At this place the viner is located at the factory and not several miles away as is often the case, hence the samples for control analyses could be taken immediately after vining and grading. About a thousand pounds of shelled Perfection peas taken from a single lot were mixed and run through a washer and grader. From these, 450 pounds of size 4 peas were taken for the experiment. The grading was done on a grader with additional washing. This operation precluded any possibility of damaging the quality of the peas by the presence of the so-called viner mucus, which might cause deterioration of the quality of the canned product (3).

The washed and graded peas were distributed, without sending them over the picking tables, into pea pails or buckets of galvanized sheet iron with numerous perforations, at the rate of about 20 pounds (9 kg) per pail. The peas in three of these pails received no cooling, those in eight pails were chilled at once, and the remainder were put through the blancher. In the blancher the peas were heated in water to 180° F. (82° C.) for five minutes. They were then cooled with tap water at 68° F. (20° C.) and chilled.

Chilling was done by placing the pails in a wooden tank containing water to a depth of some 30 cm and a considerable quantity of chopped ice, with a temperature of 39° F. (4° C.). The pails were lifted so as to drain a little and were resubmerged several times. In three minutes they reached a temperature of 43° F. (6° C.) as shown by a thermometer thrust into the peas. Equilibrium with the water temperature was attained within five minutes. As soon as the last of the blanched samples was thoroughly chilled, all were removed, covered with chopped ice, and transported by motor truck to a cold-storage warehouse at Geneva, N. Y. The samples were in the cold-storage warehouse within three hours after the arrival of the vines at the vining machine and within little more than one hour after the beginning of the vining.

At the cold-storage warehouse the pails were placed on the floor of a large room held at 30° F. (-1° C.). The temperature in the center of the pails was 38° to 40° F. (3° to 4° C.) when they were put in the cold-storage room. In two hours the temperature of the peas fell to 35° to 38° F. (2° to 3° C.), showing that the usual heating of peas in the pails (4) had been entirely avoided by the cooling and by the transportation under ice.

Samples for chemical analysis were taken after grading and washing, but before cooling. Other samples were taken from the blanched peas immediately after leaving the blancher. A canning test for control was made at once after the blanching. The cans were closed and processed as described later.

During their stay in the cold storage, no attempt was made to stir the peas or to prevent or remove the accumulated moisture. At various intervals a pail of each lot was withdrawn and the contents canned. The peas stored at outside air temperature were hot and slimy at the end of 30 hours, so only 2 cannings, 1 at 6 hours and 1 at 20 hours, could be made from these.

For a canning test the peas that had not been heated before storage (the O and C series) were blanched in water at 180° F. (82° C.) for five minutes. The previously blanched or B series were slightly warmed by flushing with tap water at about 63° F. (17° C.). The peas were then weighed at the rate of 10.5 ounces (298 g) to each can (83 mm diameter by 72 mm high inside). Hot brine containing 15 pounds of salt and 30 pounds of sugar per 132 gallons of water was poured in from the regular filling machines, which were operating at the same time, until the cans were full, and the temperature (closing temperature) of several cans was noted. The cans were then closed by regular high-speed closing machines. The cans were processed at 244° F. (118° C.) for 35 minutes and cooled in the usual way in a cold-water cooling tank for about 35 minutes at 75° to 90° F. (24° to 32° C.). The cans were stored for about two months at the experiment station at room temperature, until they were shipped to the different places to be judged and scored.

Samples for chemical analysis were taken before each canning test. The samples were preserved by boiling for 30 minutes in 80 per cent alcohol solution. In general, the methods of the Association of Official Agricultural Chemists were used (1). Exceptions to this were that sugars were determined by Bertrand's method (2) and that moisture determinations were made by drying a portion of whole peas for 48 hours at 100° C.

RESULTS OF SCORINGS OF THE CANNED PEA SAMPLES

Samples of the canned peas were submitted to the Washington office of the National Cannery Association; to the Division of Fruits and Vegetables, Bureau of Agricultural Economics, United States Department of Agriculture; to the Wisconsin Department of Markets; to Strasburger and Siegel, consulting chemists; and to a group of members of the agricultural committee of the Association of New York State Cannerymen. Numerical scorings made according to the scoring sheets adopted by the United States Department of Agriculture were obtained from all except the National Cannery Association. The scores are presented in Table 1.

TABLE 1.—Scoring of canned peas which underwent various treatments before canning

Sample No.	Code	Treatment	Storage	Scoring by agencies indicated * for—																		Total score (100 points)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																					
				Clearness of liquor (15 points)				Absence of defects (15 points)				Uniformity of size (10 points)				Tenderness and maturity (35 points)				Flavor (25 points)																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																							
				1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2			3	4	Average	Grade *																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																
1				13	13	13	14	13	13	13	13	13	8	9	7	8	29	32	31	30	21	21	21	84	88	85	86																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																																

From the Washington laboratory of the National Cannery Association only descriptive opinions were received, without numerical scoring. As flavor is the most important factor in the present experiment, the opinion of this laboratory about the flavor of the pea samples is quoted as follows:

The peas in cans PBH and POB had a flavor which we have come to associate with the deterioration of peas which have been held too long between vining and canning. The peas in cans PCE and PCF had a peculiar abnormal and objectionable flavor with which we are not familiar. None of those judges who tasted these peas had ever experienced this particular flavor in connection with canned peas and we were not able to arrive at any reasonable suggestion as to the cause of this poor flavor.

The gradings for the control samples showed an average of 86 points, which placed them in the range of Extra Standard peas. If the control pack had been Fancy, the resulting samples might have deteriorated at a different rate from the present samples.

There was a very rapid decrease in quality in the peas stored at outside temperature. The sample canned 6 hours after vining was scored 78 and declared to be Standard grade. As one would expect, the sample canned 20 hours after vining resulted in a pack of inferior quality, which was scored by all judges as Substandard, with the average score of 63.

When the blanched and cooled samples were taken to the factory for the various canning trials, they were always submitted to the factory manager who was unable to detect any change in the characteristics of the peas. This fact was not surprising, since Smith (8) also notes that the real character of the peas becomes apparent only after canning and storing. At the last cannings the peas of the C series (cooled without blanching) appeared to be in the raw state somewhat harder than the corresponding sample from the B series, which was blanched before cooling.

The blanched and cooled sample was found by all judges to be in the extra standard or standard grade after 72 hours' storage. The peas in the cans packed after 96 and 144 hours were found to be substandard by one grader (Strasburger & Siegel), but were rated as standard by the other two. The cans packed after 190 hours of storage were undoubtedly substandard. The code of this sample was PBH, and this was one of the samples mentioned by officials of the National Cannery Association as being "abnormal and objectionable." According to these scorings, one might expect the peas held in cold storage to yield packs of standard or higher grade until the period between 72 and 96 hours, while the definite change from standard to substandard packs probably occurred in the period between 120 and 150 hours after vining.

The peas cooled without blanching appeared to have deteriorated somewhat more rapidly than did those blanched before cooling. The sample canned 48 hours after vining was in the standard grade, while the sample canned 72 hours after vining gave a substandard pack. This was one of the bad-flavored samples, according to the opinion of the laboratory of the National Cannery Association. While the sample canned after being held 72 hours was scored substandard by one judge only, the next sample, canned 96 hours after vining, was declared by two of them to be in this grade. The change from the standard to the substandard grade must have occurred some time between 50 and 100 hours, undoubtedly sooner than that in the blanched and cooled sample.

TABLE 2.—Changes in the chemical composition of peas during cold storage*

[Figures are percentages on the basis of fresh wet peas]

Constituent	Percentage content of peas, not blanched before storage, after indicated hours of storage					Percentage content of peas, blanched before storage immediately after shelling, after indicated hours of storage							
	Con- trols, not stored ^a	CB, 20 hours	CD, 48 hours	CE, 72 hours	CF, 96 hours	CG, 144 hours	Con- trols, not stored ^b	BB, 20 hours	BD, 48 hours	BE, 72 hours	BF, 96 hours	BG, 144 hours	BH, 190 hours
Dry matter.....	23.15	22.83	21.63	21.75	22.26	20.64	22.39	21.74	21.69	22.36	21.15	21.97	20.03
Alcohol-soluble matter.....	6.56	7.71	6.28	7.83	7.62	5.31	5.10	4.87	5.57	5.40	4.48	5.53	4.36
Reducing sugars.....	.37	.25	.41	.05	.16	.28	.26	.13	.19	.03	.16	.05	.54
Sucrose.....	3.51	2.37	2.54	2.51	2.37	1.75	2.64	2.62	2.30	2.48	2.32	2.43	2.23
Total sugars.....	3.88	2.62	2.95	2.56	2.53	2.03	2.90	2.75	2.49	2.51	2.48	2.48	2.77
Nonprotein nitrogen.....	1.58	1.65	1.55	1.59	1.60	1.66	1.30	1.19	1.04	1.13	1.01	1.05	1.44
Protein nitrogen X 6.25.....	9.86	1.034	9.72	9.95	1.000	1.040	1.813	1.744	1.651	1.707	1.631	1.657	.903
Unidentified alcohol-soluble matter.....	1.70	4.06	2.36	4.27	4.09	2.24	1.39	1.38	2.43	2.18	1.37	2.39	.69
Total alcohol-insoluble matter.....	16.99	15.12	15.33	13.92	14.64	16.33	17.29	16.87	16.12	16.96	16.67	16.14	15.67
Crude fiber.....	3.13	2.60	2.66	2.78	2.65	2.65	3.44	3.40	2.76	3.00	2.96	3.07	2.85
Starch.....	1.93	1.86	1.72	1.48	1.66	1.77	1.94	1.85	1.84	1.96	2.09	1.78	1.79
Protein nitrogen.....	1.947	1.885	8.56	7.73	8.22	8.73	9.85	9.89	9.85	9.96	9.93	9.93	9.93
Protein nitrogen X 6.25.....	5.92	5.41	5.35	4.83	5.14	5.46	6.03	5.62	5.41	5.91	5.58	5.72	5.33
Unidentified alcohol-insoluble matter.....	5.61	5.23	5.62	4.85	5.28	5.45	5.92	6.00	6.11	6.09	5.94	5.69	5.70
Total nitrogen.....	1.105	1.030	1.011	.932	.982	.939	1.095	1.018	.969	1.058	.993	1.020	.996

* Double letters at top of columns are codes of samples.

^b Averages.

TABLE 3.—*Changes in the chemical constituents of single peas during cold storage*^a

[Figures are in grams of weight per single pea]

Constituent	Percentage of content of peas, not blanched before storage, after indicated hours of storage					Percentage content of peas, blanched before storage immediately after shelling, after indicated hours of storage							
	Con- trols, not stored ^a	CB, 20 hours	CD, 48 hours	CE, 72 hours	CF, 96 hours	CG, 144 hours	Con- trols, not stored ^a	BB, 20 hours	BD, 48 hours	BE, 72 hours	BF, 96 hours	BG, 144 hours	BH, 190 hours
Total weight.....	0.4060	0.430	0.430	0.430	0.416	0.446	0.3731	0.404	0.394	0.396	0.383	0.394	0.389
Dry matter.....	.0941	.0682	.0630	.0655	.0928	.0920	.0635	.0674	.0654	.0896	.0612	.0846	.0782
Alcohol-soluble matter.....	.0267	.0317	.0257	.0344	.0320	.0238	.0190	.0194	.0218	.0216	.0168	.0214	.0172
Reducing sugars.....	.0015	.00108	.00176	.00021	.00061	.00125	.00097	.00052	.00075	.00012	.00062	.00019	.00210
Sucrose.....	.0143	.0102	.0109	.0111	.0099	.0078	.0099	.0106	.0090	.0098	.0089	.0094	.0067
Total sugars.....	.0158	.0113	.0127	.0113	.0105	.00905	.01087	.0111	.00981	.0100	.00953	.00954	.0108
Nonprotein nitrogen.....	.0064	.00707	.00666	.00683	.00665	.00742	.00949	.009481	.009410	.009421	.009386	.009404	.00956
Nonprotein nitrogen X 0.25.....	.00401	.00442	.00416	.00427	.00416	.00464	.00503	.00501	.00538	.00563	.00552	.00550	.00550
Unidentified alcohol-soluble matter.....	.00991	.0160	.0088	.0188	.0173	.0102	.00519	.0053	.0064	.0091	.0049	.0093	.0079
Total alcohol-insoluble matter.....	.0677	.0665	.0673	.0611	.0608	.0682	.0645	.0690	.0636	.0670	.0644	.0632	.0610
Starch.....	.0127	.0118	.0114	.0105	.01085	.01182	.0128	.01370	.01087	.01188	.01124	.01179	.01109
Crude fiber.....	.0078	.00398	.00740	.00629	.00690	.00789	.0071	.00747	.00725	.00778	.00800	.00694	.00698
Protein nitrogen.....	.0085	.00342	.00370	.00384	.00342	.00390	.006	.00640	.00342	.00374	.00342	.00352	.00332
Protein nitrogen X 0.25.....	.00425	.00171	.00185	.00192	.00171	.00195	.0025	.00213	.00185	.00187	.00171	.00179	.00176
Unidentified alcohol-insoluble matter.....	.0238	.0258	.0241	.0220	.0219	.0244	.0221	.0239	.0214	.0234	.0214	.0220	.0206
Total nitrogen.....	.0045	.00413	.00437	.00402	.00409	.00464	.0041	.00388	.00383	.00416	.00381	.00392	.00388

^a Double letters at top of columns are codes of samples.

^b Averages.

RESULTS OF THE CHEMICAL ANALYSES

The samples for chemical analysis were taken immediately after grading, after blanching, and before every canning test. They were preserved by boiling in alcohol, as mentioned above, and were analyzed after two weeks of storage. The results, calculated on the basis of the fresh weight of the peas when the samples were taken, and on the basis of a single pea, and are presented in Tables 2 and 3. The weight of a single pea was determined by counting 50 g of peas used for a sample.

The dry-matter content of the samples decreased slightly during storage. The weight of a single pea, on the other hand, increased, probably because of the condensation of moisture from the air of the room on the peas. No effort was made to dry the peas before sampling. This accounts for a part of the decrease of the percentage of dry matter, which occurred in the bleached peas. This fact, however, shows the advantages of presenting the data on a single-pea basis, because in this way changes in the actual quantities of the constituents can be traced regardless of the loss or gain of moisture and gases.

There was some decrease in the total sugars in the samples of the unblanched series, the largest decrease being shown during the first 20 hours of the experiment. Blanching reduced the amount of total sugars to the same degree as did storage of the unblanched sample for 20 hours. Total sugars may be consumed by respiration in the unblanched sample and leached out in the other. The decrease in the percentage of sucrose and of the total sugars is much less than the loss of the latter constituent in peas kept at room temperature, for which the senior writer (5) showed as much as 52 per cent decrease in total sugar and starch content during the first 24 hours. The losses in the total sugars of the unblanched samples were roughly 30 per cent, and were possibly related to a small, but definite loss of flavor, namely 3 points of a possible 25. The only significance to be attached to this fact is that it indicates a certain enzymic and respiratory activity that is undoubtedly related to the more rapid deterioration of the unblanched samples.

In storage there was practically no decrease in the total sugars in the blanched samples, showing that heating for five minutes to 180° F. (82° C.) had mostly inactivated the saccharose and the respiratory enzymes.

Only immaterial changes could be found in the starch content of the peas. This shows that even in the unblanched sample amylase activity was unimportant. This enzyme was shown by Kertesz (5) to be very active in macerated peas at room temperature. Likewise, the crude-fiber content of the samples did not show any greater variation than may be regarded as due to sampling and experimental error.

The nitrogen compounds were separated into 80 per cent alcohol-soluble and 80 per cent alcohol-insoluble fractions. For the sake of comparison they were calculated as proteins (nitrogen \times 6.25). This was also necessary in the subtraction made to determine the unidentified fraction, since the actual weight of nonprotein nitrogen compounds is certainly nearer to nitrogen \times 6.25 than to the actual weight of nitrogen found. It must be understood, however, that the 80 per cent alcohol-soluble nitrogen fraction should be regarded as being composed mostly of nonprotein nitrogen compounds. Changes

in both fractions of nitrogen in the samples as analyzed were small and not greater than the experimental error.

None of the constituents determined directly appeared to fluctuate in conformity with the observed changes in quality.

DISCUSSION

The most obvious conclusion to be drawn from the present experiment is that deterioration in flavor is a more important consideration in holding shelled peas in storage before canning than texture, or the variation of the more obvious of the chemical constituents. The changes in texture, or the increase in toughness, which have been given so much emphasis recently, have a relatively minor effect on the total score and do not occur until much later than the loss of flavor.

It is also obvious that in the present investigation the changes in the flavor of peas held after vining can not be explained by reciprocal conversions of sugar and starch. The statement that sugar is changed to starch during holding is often encountered in the trade literature concerning peas, but in this experiment no evidence of such a change was found. This should not appear startling, for the flavor of the peas is judged after canning in a solution containing sucrose. Furthermore it has been found that the addition of the indicated sugar does not make the flavor of lower-grade peas equal to that of choice peas. In the unblanched samples, where the enzymes had not been inactivated, there was a slight decrease in the absolute amount of starch (single pea basis), together with some loss in the total sugars as well. It is suggested that respiration accounts for this loss of starch and sugar.

It is reasonable to suppose that the respiration in the unblanched samples was not entirely stopped by cooling, and this would account for the more rapid deterioration of these peas. Table 1 shows that the blanched peas held for more than 72 hours were always scored higher than were the unblanched. The small extent of the changes in the constituents determined chemically is in sharp contrast with the great extent of the changes in the flavor.

The changes observed in the amounts of the two nitrogen fractions are insignificant. An alteration in the proportions of the alcohol-soluble and the alcohol-insoluble fractions during storage was expected, but only immaterial changes could be found. It was supposed that the sharp flavor often encountered in peas held too long after vining might be accounted for by some such change. No sharp flavor was developed in these samples, and therefore this hypothesis could not be tested.

The question still remains, why did not the combination of blanching and cooling prevent the deterioration of the peas? Two suggestions are offered as possible answers. One is that the changes in the peas held in cold storage may be caused by direct oxidation by the oxygen of the air, independently of respiration or other enzyme actions. These changes could then possibly be eliminated by storing the peas in nitrogen or carbon dioxide. Because of the very short season for obtaining material this supposition could not be tested this year. The second suggestion follows from the fact that, as Kertesz (5) has shown, all the chemical changes proceed more rapidly

in macerated peas than in the normal whole peas. It is well known that the rate of respiration increases in bruised or injured plant tissues. When peas are shelled by machine in the usual way, and then separated into sizes over rolling or vibrating metal screens a greater or less amount of bruising is inevitable. This bruising is equivalent in its effect to a certain amount of maceration, and such peas have been shown by Sayre, Willaman, and Kertesz (6) to deteriorate more rapidly than do hand-shelled peas. The changes in the machine-shelled peas proceed with extreme rapidity so that they become far advanced during the short interval between shelling and cooling. These changes may then be retarded or stopped altogether by blanching or cooling, but they are already extensive enough to produce a decidedly deleterious effect on the flavor. Apparently, blanching will stop these changes better than does cooling alone. The condition reached after blanching is then maintained for a longer period than that of peas simply chilled, where respiration is probably at a higher rate.

SUMMARY

Peas were kept in cold storage for several days without deterioration when chilled quickly to the storage temperature (30° F.). Deterioration was slower in peas blanched before cooling than in peas cooled and stored at 30° without blanching.

During storage the percentage and absolute amount of total sugars in the unblanched, chilled sample decreased, showing respiratory activity. In the blanched and chilled peas there was no loss in sugars, showing that the respiratory enzymes were mostly inactivated by heating the peas for five minutes to 180° F.

The percentage and absolute amount of starch per pea did not increase in shelled and stored peas, showing that there was no conversion of sugars to starch.

None of the chemical constituents determined appeared to fluctuate in conformity with the observed changes in quality.

LITERATURE CITED

- (1) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1925. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. REVISED TO JULY 1, 1924. Ed. 2, 535 p., illus. Washington, D. C.
- (2) BERTRAND G.
1906. LES DOSAGES DES SUCRES RÉDUCTEURS. Bul. Soc. Chim. France 35:1285-1289.
- (3) BURR H. R.
1930. ANNUAL REPORT OF THE EXECUTIVE SECRETARY. Canning Trade 53 (13):12, 14, 18.
- (4) GOWEN, P. L.
1928. EFFECT ON QUALITY OF HOLDING SHELLED PEAS. Canner (Convention Number) 66:112, 115.
- (5) KERTESZ, Z. I.
1930. THE CHEMICAL CHANGES IN PEAS AFTER PICKING. Plant Physiol. 5:399-412, illus.
- (6) SAYRE, C. B. WILLAMAN, J. J., and KERTESZ, Z. I.
1931. FACTORS AFFECTING THE QUALITY OF COMMERCIAL CANNING PEAS. N. Y. State Agr. Expt. Sta. Tech. Bul. 176, 76 p., illus.
- (7) SMITH H. R.
1930. SPECIAL INVESTIGATIONS. EFFECT OF HOLDING VINE PEAS IN COLD STORAGE. Canner (Convention Number) 70:211-212.

LIFE HISTORY AND HABITS OF CRESTED WHEATGRASS¹

By L. DUDLEY LOVE, formerly Assistant, Department of Botany, and HERBERT C. HANSON, Botanist, North Dakota Agricultural Experiment Station²

INTRODUCTION

Crested wheatgrass, *Agropyron cristatum* J. Gaertn., has recently become important as a pasture and hay plant in the northern part of the Great Plains as well as in the Rocky Mountain region. It is especially valuable because it begins to grow a week or two earlier than other grasses in the spring. It is an upright perennial bunch grass and is well adapted to many of the lighter soils in the semiarid regions.

L. E. Kirk,³ in a bulletin recently published, has described the characteristics of crested wheatgrass that adapt it to cold and drought. He has shown that it is successful in controlling weeds, and he has discussed its uses as hay and pasture and as turf for lawns and golf courses.

THE SEED

The seed is light yellow in color, wide and blunt at the base, and has a short awn. The lemma and palea are prominently rolled. The back and sides of the lemma contain several well-spaced spines toward the tip. (Fig. 1.) The rachilla is not appressed to the lemma and palea. *Agropyron cristatum* is easily distinguished from both *A. tenerum* Vasey and *A. smithii* Rydb. by its smaller size and rolled shape.

The rachilla segment (fig. 2) of *Agropyron cristatum* has a wide blunt base and U-shaped sinus, the lemma edges at the callus not obscuring the almost parallel sides of the rachilla. The apex of the rachilla is oblong and the segment itself is characterized by prominent stout hairs.

The palea is hirsute over the entire surface. The hairs on the edge of the palea are short, stout, somewhat pointed and spaced farther apart than those of *Agropyron tenerum* or *A. smithii*.

Key for Identification of Four Species of *Agropyron*

Rachilla cone-shaped, arising from a V-shaped sinus; apex elliptical.

Rachilla segment hirsute. Palea hirsute over entire surface; hairs on edges stout and fairly long.....*Agropyron smithii*

Rachilla segment pilose. Palea puberulent at tip; hairs on edges fine, acute, and close together.....*Agropyron tenerum*

Rachilla cylindrical in shape, arising from a U-shaped sinus; apex oval to oblong.

Apex of rachilla segment heart-shaped; segment hispidulous. Palea puberulent at tip; hairs on edges short, stout, and blunt.....*Agropyron repens*

Apex of rachilla segment oblong; segment sparsely hispid. Palea hirsute over entire surface; hairs on edges short, stout, somewhat pointed, and spaced fairly far apart.....*Agropyron cristatum*

¹ Received for publication Jan. 13, 1932; issued October, 1932.

² The authors are indebted to Prof. O. A. Stevens for the facilities necessary to make the germination tests and to J. T. Sarvis, Leroy Moomaw, and Samuel Garver for supplying seed.

³ KIRK, L. E. CRESTED WHEAT GRASS. Saskatchewan University Agr. Ext. Bul. 54, 22 p., illus. 1932.

In order to determine the length of time that crested wheatgrass seed retains its viability, a series of germination tests were made.

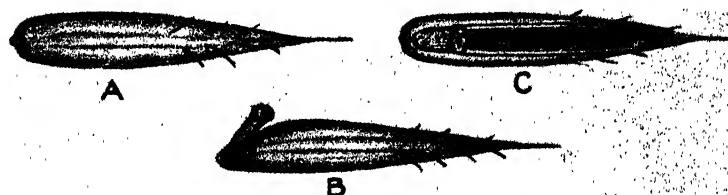


FIGURE 1.—Dorsal (A), lateral (B), and ventral (C) view of the seed of crested wheatgrass

One hundred and fourteen lots of seed grown in various years from 1919 to 1930 were tested. The seed came from Mandan, N. Dak., Dickinson, N. Dak., Redfield, S. Dak., Havre, Mont., and Saskatoon, Canada. Several of the lots were individual plant selections made by J. T. Sarvis or A. C. Dillman. The seed in most cases had been stored in a dry, moderately cool place. Each lot of seed was tested once in the period from October to November, 1930, in accordance with the methods of the official seed analysts. One test was considered sufficient when averaged with other lots of the same year.

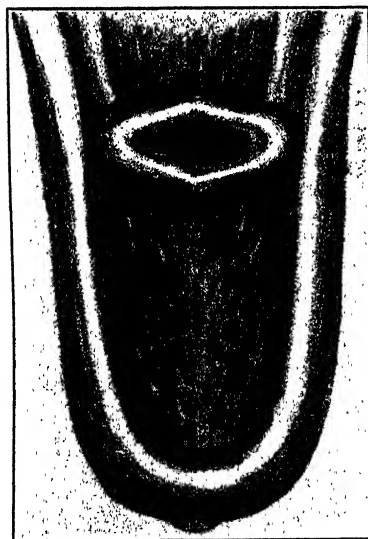


FIGURE 2.—Rachilla segment of crested wheatgrass

The number of lots that were tested, and the average percentage of germination are presented in Table 1. This table shows a sharp decline in the percentage of germination of seed harvested prior to 1926. It was noted in the germination tests that the 1930 seed germinated more completely at the end of five days than the 1926 or 1927 seed. This fact suggests that the rate of germination decreases along with the percentage of germination. The data show, then, that crested wheatgrass seed, if stored in a cool, dry place, may be expected to germinate satisfactorily until 4 years of age.

TABLE 1.—Percentage germination of crested wheatgrass seed of various years

Year	Lots	Average germination	Year	Lots	Average germination	Year	Lots	Average germination
	Number	Per cent		Number	Per cent		Number	Per cent
1919	10	1.0	1923	22	30.6	1927	2	85.5
1920	15	5.5	1924	2	32.0	1928	0	—
1921	2	0	1925	4	53.0	1929	2	90.0
1922	1	4.0	1926	28	56.1	1930	28	91.2

DEVELOPMENT AND MORPHOLOGY OF THE SEEDLING

In its natural state crested wheatgrass seed falls to the ground shortly after maturity and lies there until favorable conditions permit germination. This indicates that the natural time of seeding is in the fall, but tests in some places show that no appreciable difference exists between spring and fall sowing.

During the fall and winter of 1930-31 tests on depth of planting were conducted in the greenhouse. Good tilth of the clay soil was secured in order to have about the same amount of moisture throughout. Two hundred seeds were planted at each of the following depths: One-eighth, one-fourth, one-half, three-fourths, 1, 2, 3, 4, 5, and 6 inches. In the bench where the plantings were made the soil was about 20 inches deep. At the end of 5, 10, and 28 days the seedlings that appeared above the surface were counted. After 28 days the leaf area of all the seedlings was determined by multiplying the average length by the average width in millimeters. The results are given in Table 2.

TABLE 2.—*Effect of various depths of planting on crested wheatgrass seed*

	Values for depths of seeding (inches) indicated						
	One-eighth	One-fourth	One-half	Three-fourths	1	2	3
Seeds.....number.....	200	200	200	200	200	200	200
Plants in 5 days.....do.....	15	4					
Plants in 10 days.....do.....	134	140	114	125	93	91	10
Plants in 28 days.....do.....	139	135	116	130	99	78	19
Average height in 28 days.....inches.....	6.5	6.5	6	6	5.3	5.5	4
Total leaf area.....cm ²	2,546.2	2,353.4	2,233.7	2,360.8	1,540.9	1,095.7	130.9
Average leaf area per plant.....cm ²	18.3	17.4	19.2	18.2	15.6	14.1	6.9
Shoots above surface in 10 days.....per cent.....	67.0	70.0	57.0	62.5	46.5	45.5	5.0
Shoots above surface in 28 days.....do.....	69.5	67.5	58.0	65.0	49.5	39.0	9.5

Cm² is the abbreviation for square centimeter recently adopted by the Style Manual for U. S. Government Printing.

The measurements taken at the end of 28 days showed a gradation in height from 6.5 inches for plants from seeds planted at the $\frac{1}{8}$ -inch depth to 4 inches for plants from seeds planted at the 3-inch depth. The difference in the average leaf area between the seedlings from the one-eighth and the one-half inch depths may have been due partly to competition, since there were fewer seedlings at the one-half inch depth than at the one-fourth or one-eighth inch depths. The maximum depth from which seedlings appeared above the surface was 3 inches. Seed of crested wheatgrass germinates well upon the surface of the soil when there is sufficient moisture, but in the field, where the surface is usually dry, it appears that the best depth at which to plant the seed is between one-fourth and one-half inch, depending upon the dryness of the surface soil.

A similar experiment for brome grass showed that the maximum depth at which the seed germinated and sent shoots to the surface was 3 inches. At this depth about 5 per cent of the seeds were able to send shoots to the surface. About 65 per cent of the seeds planted at one-eighth to 1 inch produced seedlings above the surface.

In order to study the development of the seedling, seeds were germinated in a damp chamber at a temperature of about 25° C. At

the end of 24 to 48 hours the coleorhiza had formed and several distinct root hairs were already present on it. At the end of 48 to 72 hours, the coleoptile began to appear. The primary root was about twice as long as the coleorhiza and had several root hairs. At 72 to 96 hours, both the coleoptile and primary root were well developed; the latter was about as long as the seed and was covered with a thick mat of root hairs. At 96 to 120 hours, the coleoptile was about as long as the seed and the primary root about twice as long as the seed. (Fig. 3.)

In a similar experiment brome grass did not develop a shoot until 48 to 72 hours after the seed was placed in a damp chamber. This indicates that, although a little slower in producing the coleoptile, it had good roots to begin shoot growth.

During the winter of 1930-31, greenhouse studies were made of the rate of root and shoot elongation of crested wheatgrass as compared with that of brome grass. This comparison was made because it had been observed that the seedlings of crested wheatgrass were much more frail and delicate than those of brome grass.

Examinations were made at the end of 10, 17, 24, 31, and 45 days. The development of the seedlings of both of these grasses is shown in Figure 4. During the first 24 days brome grass had a distinct advantage over crested wheatgrass in both root and shoot development. Although the main root of brome grass penetrated somewhat deeper, the branches were fewer. At all stages the height of the shoot in both grasses was approximately the same, but the leaves of brome grass were wider. Brome grass therefore had a larger and more vigorous seedling.

TABLE 3.—Root and shoot development of seedlings of crested wheatgrass and brome grass at different ages

Age intervals (days)	Crested wheatgrass				Brome grass			
	Average daily growth in height		Average daily growth		Average daily growth in height		Average daily growth	
	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root
	Inches	Inches	Per cent	Per cent	Inches	Inches	Per cent	Per cent
10 to 17.....	0.357	0.107	8.92	4.28	0.286	0.250	7.14	6.66
17 to 24.....	.214	.143	3.89	4.08	.286	.250	4.76	4.54
24 to 31.....	.214	.29	3.56	5.19	.143	.143	2.04	2.19
31 to 45.....	.214	.160	2.24	2.06	.178	.178	1.87	1.97

In order to determine whether crested wheatgrass developed more rapidly after a certain period than brome grass, the following method was devised: The height or length, measured in inches at the end of an interval (i. e., 17 or 24 days, etc.), was divided by the number of days in the interval in order to secure the average daily growth in height. This average daily growth, divided by the average maximum height at the end of the interval, multiplied by 100, gives the average daily height percentage that the plant grew each day during a particular interval. Table 3 shows the root and shoot development of seedlings of crested wheatgrass and brome grass. From these data it appears that the development of both the root and shoot is more rapid in brome grass than in crested wheatgrass during the first 24

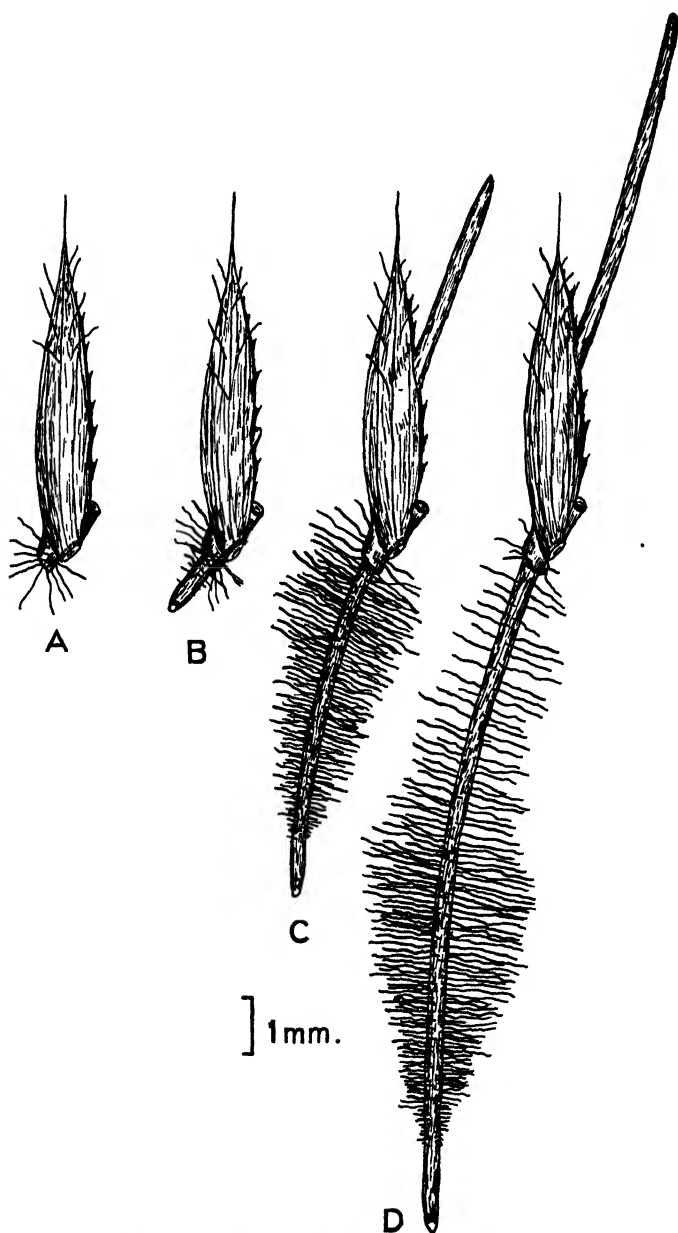


FIGURE 3.—Development of crested wheatgrass seedling: A, When aged 24 to 48 hours; B, 48 to 72 hours; C, 72 to 96 hours; D, 96 to 120 hours

days. From then on to the end of 45 days, the development of the root and shoot of crested wheatgrass is slightly more rapid than that of brome-grass. This is offered as a possible explanation of how crested wheatgrass overcomes the handicap of a very small seedling and develops into one of the most drought resistant of forage plants. This change during the seedling stage has also been noted in the field.

Very young seedlings of crested wheatgrass present a very difficult problem in identification. There are a few characters, however, that appear to be reliable for identification during the first 31 days after planting, or until there is a definite formation of the vegetative characters similar to those of the mature plant.

At the end of 10 days after planting, the number of veins in the first leaf was almost always three. The coleoptile was often greenish pink in color. The first leaf was about 0.75 mm wide, green, curled,

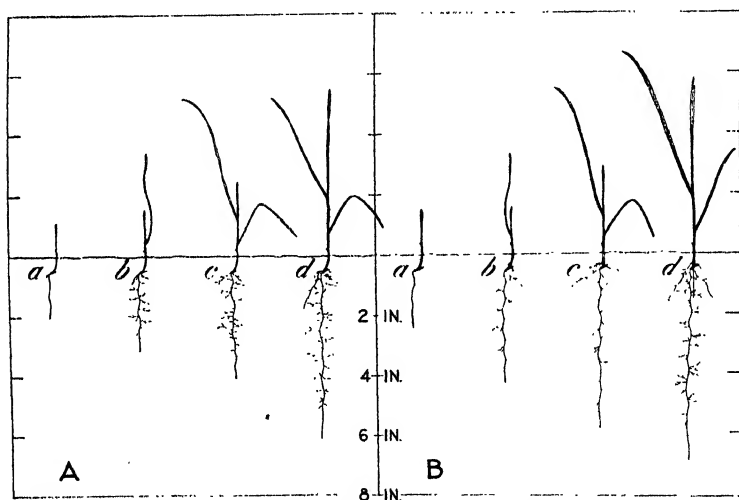


FIGURE 4.—Comparison of the development of root and shoot of crested wheatgrass (A) and brome-grass (B): a, 10 days after planting; b, 17 days after planting; c, 24 days after planting; d, 31 days after planting

and somewhat convolute. At the end of 17 days after planting, the first leaf still had three veins and was about 1 mm wide. There were a few hairs along the veins. Twenty-four days after planting, the first leaf had the same characteristics as those mentioned above, except that sometimes there appeared to be a slight development of auricles. The second leaf had five veins, was green, and about 1.25 mm wide. There were several hairs along the veins. The ligule, collar, and sheath resembled those of the mature plant and the auricles were slightly developed. The third leaf was light green, convolute, 1 mm wide, and had from three to five veins. There were a few hairs along the veins. Thirty-one days after planting, the coleoptile began to slough off. The first leaf had not changed and the second leaf had changed but slightly. The latter was 1.5 mm wide and the auricles were much more pronounced. The third leaf was green and about 2 mm wide. It had about six veins and several hairs along the veins.

Ten-day-old seedlings of crested wheatgrass may be distinguished from those of brome grass by the width of the leaf, the number of veins, and the hairiness. From the seventeenth day after planting crested wheatgrass may be distinguished by the characteristic ligule, collar, and auricles.

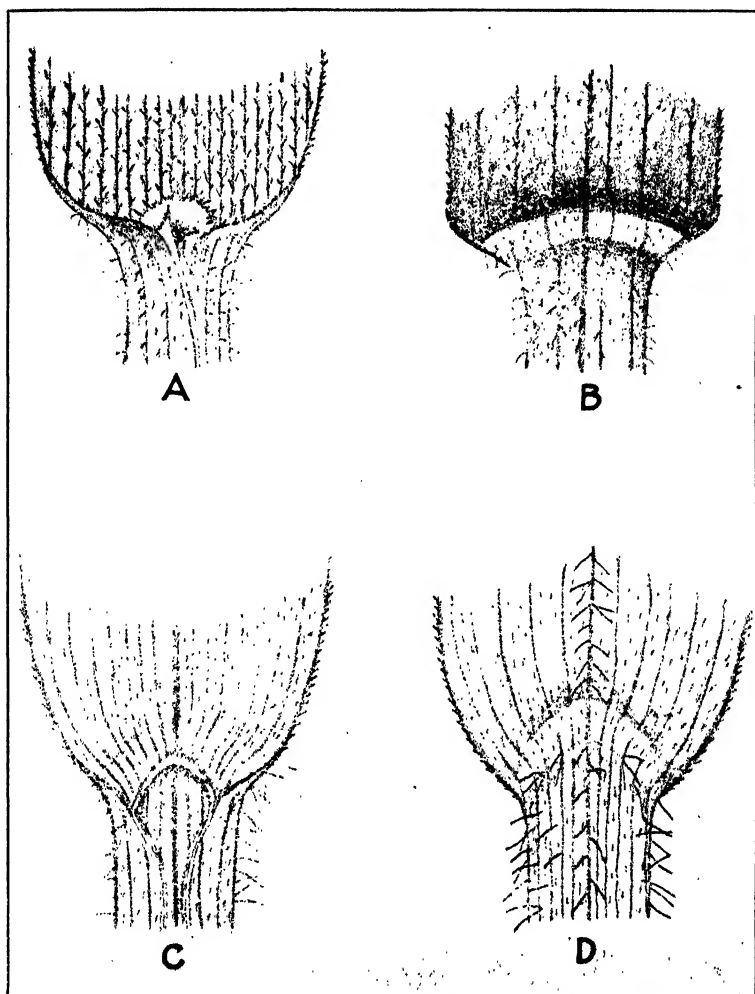


FIGURE 5.—Base of mature blades, showing sheath, collar, and ligule of crested wheatgrass (A, B) and brome grass (C, D)

DEVELOPMENT AND MORPHOLOGY OF THE MATURE PLANT

Crested wheatgrass is a perennial of bunch-grass habit; 20 to 30 inches high; leaves rolled in the bud; collar broad, somewhat hairy; auricles slender, clawlike; ligule membranaceous, short, much lacerated; sheath not compressed, somewhat hairy; blades flat to convo-

lute, usually stout hairs along the veins, 0.12 to 0.25 inch wide, somewhat tapering to a slender, sharp point; nerves prominent on upper surface. (Fig. 5.)

From one and one-half to two months after planting, crested wheat grass begins to tiller. New stalks arise from buds on the basal node. The crown increases in size in this manner, very often exceeding several square inches in area. From nodes on old tillers or secondary stems, new groups of tillers arise, as shown in Figure 6.

In the spring, the new growth begins both from the base of old stalks and from new buds arising at nodes. Very often these new

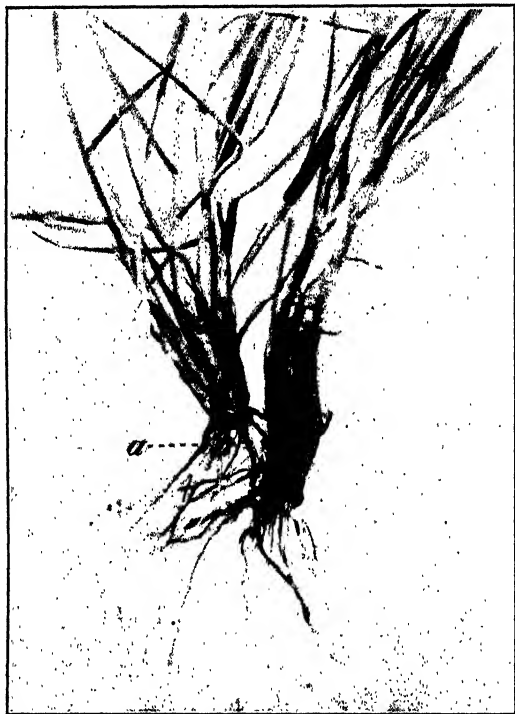


FIGURE 6.—Method by which clumps of crested wheatgrass enlarge. Note tillering from the secondary stem (a) and also from the primary stem below

shoots appear above the surface several inches away from the crown. The culms are erect and under favorable conditions will produce a spike, but under unfavorable conditions the apex ends in a mass of false leaves.

In the latter part of October, 1930, the root systems of crested wheatgrass and brome grass were excavated by the trench method. Both plots in which plants were growing had been seeded in the spring of 1929 by the Department of Agronomy of the North Dakota station. The soil is Fargo clay⁴ (alkali phase) and includes three

⁴ KNOBEL, E. W., PEIGHTAL, M. F., and CHAPMAN, J. E., SOIL SURVEY OF CASS COUNTY, NORTH DAKOTA. HOPPER, T. H., and WALSTER, H. L. PART 2: THE CHEMICAL COMPOSITION OF THE SOILS OF CASS COUNTY, U. S. Dept. Agr., Bur. Chem. and Soils, Soil Survey Rpt. No. 29, Series 1924, 80 p., illus. [1929.]

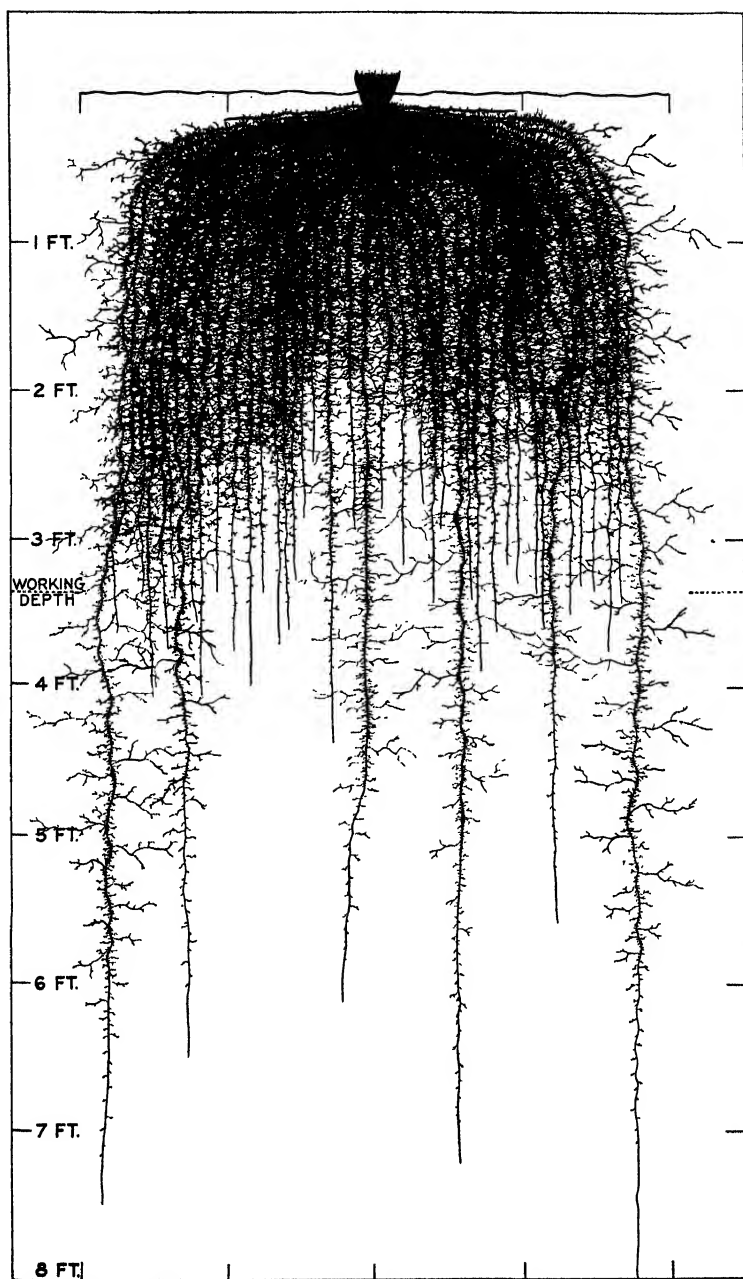


FIGURE 7.—Root system of crested wheatgrass at the end of the second season, one-fourth of the total roots being shown. Fargo, N. Dak., October, 1930

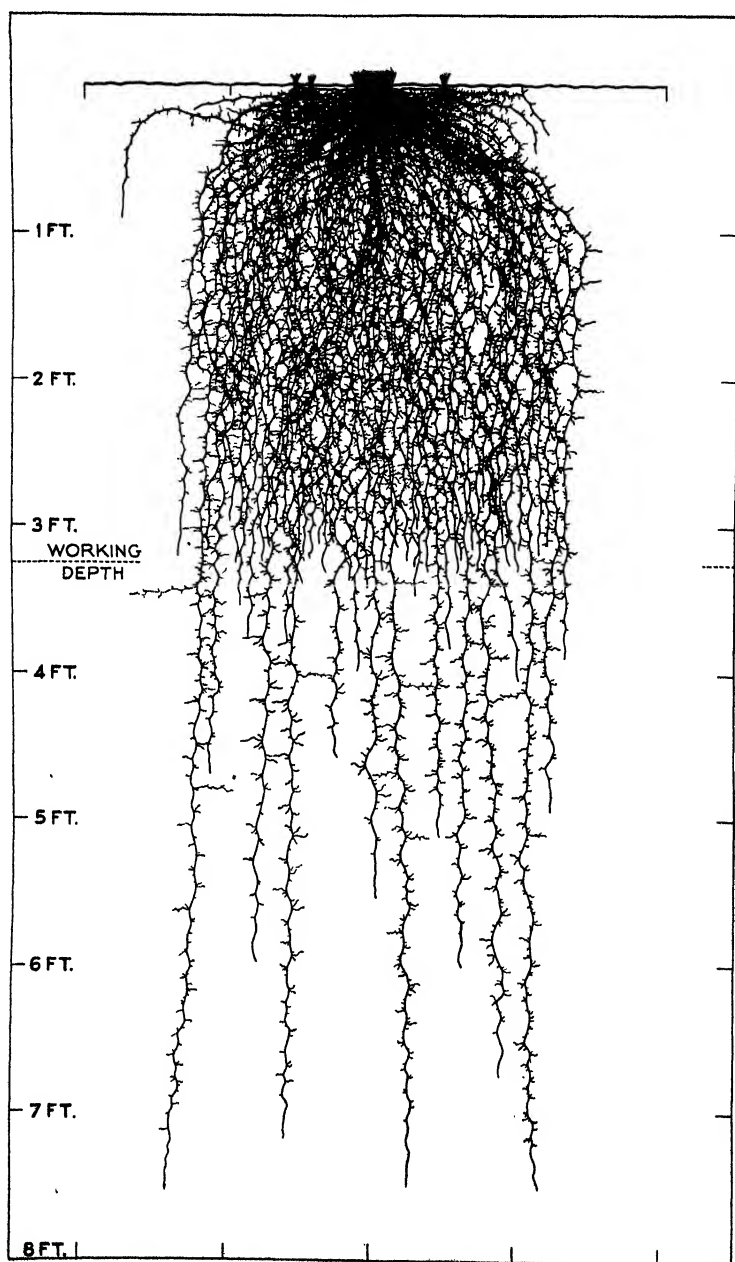


FIGURE 8.—Root system of bromegrass at the end of the second season, one-fourth of the total roots being shown. Fargo, N. Dak., October, 1930

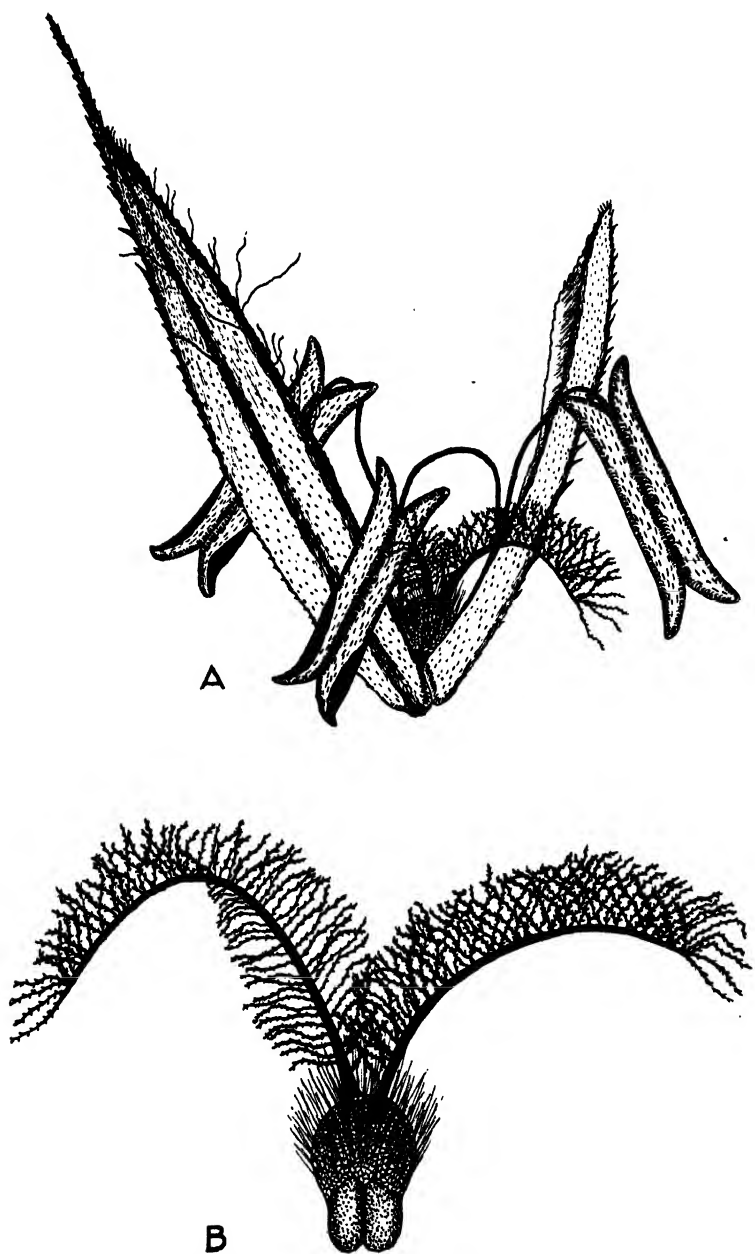


FIGURE 9.—Floret (A) of crested wheatgrass, with an enlarged style and ovary (B)

distinct horizons. The A horizon consists of a black clay to a depth of 19 inches; the B horizon is composed of a grayish-drab or olive-gray clay, containing scattered concretions of lime carbonate, and extends to a depth of 32 inches; the C horizon consists of an olive-gray or light olive-gray clay to a depth greater than 8 feet.

Crested wheatgrass (fig. 7) penetrated to a maximum depth of 8 feet, with a lateral spread from the crown of a little over 2 feet. A majority of the roots extended to a depth of 3.3 feet (working level). The root system as a whole was in excellent condition. The main roots ranged from 0.4 to 1.5 mm in diameter. The light-colored main roots, arising from 1 to 2 inches below the soil surface gave rise to numerous secondary and tertiary rootlets from near the surface to within 3 inches of the tip. The ratio of the maximum depth to the lateral spread was 3.6. The lime carbonate in the B horizon, appeared to have little effect on the color or growth of the main roots,

Bromegrass (fig. 8) penetrated to a maximum depth of 7.5 feet with a lateral spread of a little over 1.5 feet. A large number of the roots extended to the working depth of 3.2 feet. The main roots, arising directly beneath the soil surface, were pale straw-colored in the A horizon and dark brown in the B and C horizons. Several roots, after striking the B horizon, grew horizontally for about 1 inch and then turned downward. Many secondary rootlets were swollen to about twice normal size at the tips between depths of 2 to 4 feet.

A few dead roots were found, some extending to a depth of 3 feet. Where the lime carbonate deposits were shallower, the working depth was found to be 3 feet. The ratio of the maximum depth to the lateral spread was 5.0.

The root systems of crested wheatgrass and bromegrass are similar in that they penetrate to about the same depth and have about the same working level. On the whole, the main roots of crested wheatgrass are lighter in color and somewhat stouter. Crested wheatgrass has a much greater number of branch roots than bromegrass and they are not so coarse as those of brome. The absorbing surface of crested wheatgrass appears to be much greater than that of bromegrass. The lateral spread of the roots of crested wheatgrass is greater than that of brome.

INFLORESCENCE

The spikes of crested wheatgrass begin to develop from two to three months after the plant commences growth in the spring. At the time of blooming the style appears shortly before the stamens are mature and the anther sacs have broken. In Figure 9 is shown a floret shortly after the anther sacs have opened. An enlargement of the styles and ovary is also shown. The lodicules are quite large and are just beneath the base of the ovary. The long hairs on the ovary are conspicuous. Both the lemma and palea are provided with numerous short, stout hairs. On both sides the lemma has one distinct nerve down the middle, parallel with the two outside nerves. Several long hairs are seen at various points on the lemma.

SUMMARY

The seed of crested wheatgrass may be distinguished from the seeds of *Agropyron repens*, *A. smithii* and *A. tenerum* by its rolled lemma and palea, its wide blunt base, and the characteristic oblong shape of the apex of the rachilla segment.

Crested wheatgrass seed when stored in a cool, dry place may be expected to retain its viability with but little deterioration for four years.

Experiments on the depth at which crested wheatgrass seed should be planted show that the optimum is from one-fourth to one-half inch, varying with the dryness of the surface soil.

Crested wheatgrass seedlings grow much more slowly during the first 24 days than brome grass seedlings. From that time on crested wheatgrass seedlings develop just as rapidly, or even more rapidly, than brome grass seedlings.

Very young seedlings of crested wheatgrass may be distinguished by a number of characters such as the greenish-pink color of the coleoptile, the three veins in the first leaf, and slenderness of the entire plant.

The method by which the crowns of crested wheatgrass enlarge is described.

The root system of crested wheatgrass at the end of the second season extended to a depth of 8 feet, with a lateral spread from the crown of a little over 2 feet. The working level was about 3.3 feet. The entire system had very many fine secondary and tertiary branches.

The flower is described. The styles appear before the stamens are mature.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 45

WASHINGTON, D. C., OCTOBER 1, 1932

No. 7

QUANTITY OF MILK OBTAINED FROM AMPUTATED COW UDDERS ¹

By W. W. SWETT, *Senior Dairy Husbandman*; FRED W. MILLER, *Senior Veterinarian and Physiologist*; and R. R. GRAVES, *Chief, Division of Dairy Cattle Breeding, Feeding, and Management, Bureau of Dairy Industry, United States Department of Agriculture* ²

INTRODUCTION

For several years the Bureau of Dairy Industry, United States Department of Agriculture, has been carrying on research work in milk secretion for the purpose of learning not only when and how milk is secreted, but to what extent abundance of secretion is associated with the history of the udder's growth and development, and with its external and internal characteristics. The results obtained by hand milking the amputated udders of two cows slaughtered at the regular hour of milking, were reported in a previous publication (9).³ The milk was drawn in two separate milkings, one as soon as possible after the amputation of the udder, the other four hours later. One of these udders yielded 85 per cent and the other 49 per cent of the average quantity obtained at corresponding milkings before death. The smaller proportion of milk obtained from the second amputated udder was attributed to rigor mortis and excessive excitement and struggling of the cow at the time of death. The results reported offered convincing evidence that milk secretion is to a great extent a continuous process, that a large proportion of the milk obtained at a milking is stored within the udder before milking is commenced, and that the capacity of the secretory system of a cow's udder is greater than the volume of milk obtained at a milking. These deductions were distinctly contradictory to the belief, which for a long time had persisted rather generally among teachers and other professional men in dairy cattle and veterinary work, that the capacity for storing milk in the cow's udder is not more than a half pint to each quarter, and that consequently nearly all of the milk obtained at any milking must have been secreted during the process of milking. The manipulation of the udder and teats was supposed to stimulate the rapid secretion of milk during this brief period.

DISCUSSION OF RECENT WORK OF OTHER INVESTIGATORS

Since the report covering the results obtained with the first two cows was submitted for publication, several investigators have reported results on work of a similar nature. Gaines and Sanmann (1), by

¹ Received for publication Feb. 4, 1932; issued October, 1932.

² Acknowledgment is made to T. E. Woodward, Senior Dairy Husbandman, Bureau of Dairy Industry, for helpful suggestions in the conduct of this work.

³ Reference is made by number (italic) to Literature Cited, p. 400.

chemical analysis of amputated udders and their contents, from cows slaughtered at the regular milking time, recovered an amount of lactose equal to that obtained in the milk from the same udders at corresponding milkings before death. Three amputated udders were included in their study. One of the udders and its contents was analyzed as a unit. The other two udders were milked by hand, and the milk so obtained was analyzed separately from the udder with its residual contents. The lactose found in the three amputated udders was, respectively, 100, 96, and 118 per cent of the quantity found by analysis in the corresponding ante-mortem milkings. These results led them (1, p. 701) to infer that—

* * * there is present in the udder at milking time an amount of milk in excess of the amount secured upon milking. Very roughly, one-fourth of the milk is obtainable by milking the amputated udder, another fourth by drainage from the sliced udder, while the remaining half is tenaciously held even in the finely ground tissues.

Gowen and Tobey (2, 3) conducted a similar experiment based on a determination of the quantity of lactose in the udder and its contents at the time of the death of the cow. They concluded that at the time of milking 80 to 85 per cent of the milk is present in the udder of a cow milking up to 30 pounds a day, and that the amount which may be secreted during milking is about 10 to 15 per cent, but can not be more than 20 per cent of the yield of the cow. Another interesting result noted is the failure to recover significant quantities of milk by subjecting the udder tissue to a pressure of as much as 2,000 pounds. In 16 out of 20 cases, the quantity of lactose found in the amputated udder was more than sufficient to account for the total lactose produced by the cow at a corresponding ante-mortem milking. The average for the 20 udders showed an excess of lactose equivalent to 2.1 pounds of the average milk produced by these cows. This was assumed to represent the quantity of milk remaining in the udder after it was believed to have been milked dry. They conclude (3, p. 127) that cows producing up to 30 pounds of milk at one milking "have the lactose equivalent of all this milk in the udder when milking commences." The conclusions of Gowen and Tobey are greatly strengthened by data which show that the nonlactating udder does not contain any lactose.

Ragsdale and his associates (7) obtained 69 per cent of the previous yield of milk from an udder of one cow after she had been killed. In a second experiment the amputation of the udder was followed by six post-mortem milkings at half-hour intervals. The total quantity of milk recovered by post-mortem milking was 11.3 pounds as compared with an average of 10 pounds for the nine days before death. A later report (8) gave results obtained with two additional cows; the quantities of milk recovered from the two amputated udders were, respectively, 36.5 and 57.96 per cent of the quantities obtained at corresponding ante-mortem milkings.

Petersen (5, p. 143) reports results obtained by milking six amputated udders, and concludes:

1. That the udder of the cow can and does contain all of the milk drawn at a milking.
2. In all probability the milk is secreted at a more or less uniform rate in the interim between milkings.
3. That post-mortem milks are normal in all respects except their fat content, which is low.

In a later report Petersen, Palmer, and Eckles (6) describe their work somewhat more fully. For five of the six cows, the right half of the udder was milked immediately before slaughter and the left half was milked post mortem.

According to the writers' conception, "milk secretion" is the process carried on by the secretory cells of the udder in removing certain constituents from the blood and converting them into milk. Milk secretion is distinguished from the act of "giving down" or expelling the secreted product. The act of "giving down" the milk varies greatly with different cows and probably may be stimulated and accelerated by the manipulation of the udder during milking. If the manipulation stimulates release or expulsion of milk already secreted, in all probability it would affect the half of an udder not milked before slaughter at least as much as the half milked immediately before slaughter. This being so, it should result in more ready removal and a relatively greater post-mortem recovery than otherwise. The results for the five cows handled in this manner by Petersen and his associates show that the average recovery of milk from the left half of the amputated udder exceeded by 5 per cent the quantity obtained from the right half immediately before slaughter. However, a comparison of the average quantity of milk obtained post mortem with that obtained from the same half of the udder at corresponding ante-mortem milkings shows a relative post-mortem recovery of only 77.8 per cent when the five cows are considered, and 80.3 per cent when the six cows are included.

The low production of the cows studied by Petersen and his associates is noted also. The average quantity of milk obtained from the left half of the six udders at corresponding ante-mortem milkings was 1,981 g, or 4.37 pounds. The average quantity obtained from the same half of the same six udders after death was 1,590.3 g, or 3.51 pounds. Almost identical production of the two halves of the udder in ante-mortem milkings of the six cows is shown. This would indicate for both halves of the six udders an average ante-mortem production of 8.74 pounds and an average post-mortem production of 7.02 pounds.

Olson and Copeland⁴ refer to unpublished data which in some respects are in accord and in other respects in contrast to those obtained elsewhere. Four cows of varying production were studied in a somewhat similar manner. Each of these cows was milked twice daily while the ante-mortem producing level was being determined. Cow No. 1 was producing 46.67 pounds of milk daily before slaughter and yielded on two post-mortem milkings 13.1 and 1.3 pounds, a total of 14.4 pounds, which was about 62 per cent of the ante-mortem production at a corresponding milking. Cow 2 was producing an average of 6.88 pounds daily. Nearly 24 hours after the last ante-mortem milking she was killed, and 2.61 pounds was drawn from one half of her udder. Cow 3 was yielding an average of 8.85 pounds daily. Nearly 24 hours after the last ante-mortem milking she was slaughtered. Just before death 3.72 pounds of milk had been removed from one half of her udder. Immediately after death the other half of the udder yielded 2.51 pounds. The writers' comment relative to a

⁴ OLSON, T. M., and COPELAND, L. Unpublished data on file at the South Dakota Agricultural Experiment Station.

similar plan employed by Petersen and his associates applies to the results obtained with this cow. Cow 4 was yielding an average of 20.61 pounds daily. By continuous massaging and milking of the udder for 1½ hours after death 8.75 pounds was obtained. Because of the lack of uniformity in the handling of these cows, a measure of the relation between the quantities of milk obtained by ante-mortem and post-mortem milking is difficult. It appears, however, that the post-mortem recovery for the four cows was 61.70, 75.87, 56.72, and 84.87 per cent, respectively, and averaged approximately 70 per cent.

DESCRIPTION OF EXPERIMENTS AND RESULTS OBTAINED WITH FOUR COWS

The methods used and the results obtained in studying the first two cows (Nos. 459 and 292) were fully described in a previous report (9). After the preparation of that report and before its publication two other cows (Nos. 123 and 272) were studied similarly. (Table 1.)

TABLE 1.—Quantity of milk obtained before death of the cow and after death and amputation of the udder

[Cows in Group 1 felled by a blow on the head and udders permitted to become chilled after death; cows in Group 2 shot and udders held at blood temperature]

Group and cow No.	Average production for 10 days, ante-mortem milking	Milk from first post-mortem milking	Milk from second post-mortem milking	Total post-mortem milk	Percentage of ante-mortem production obtained in post-mortem milkings
	Pounds	Pounds	Pounds	Pounds	
Group 1:					
459.....	12.07	9.20	1.07	10.27	85.09
292.....	21.38	7.70	2.90	10.60	49.58
123.....	10.37	5.90	1.30	7.20	69.43
272.....	21.83	6.50	2.30	8.80	40.31
Average.....	16.41	7.33	1.89	9.22	61.10
Group 2:					
846.....	18.51	15.40	3.30	18.70	101.03
903.....	16.27	9.00	2.60	11.60	71.30
811.....	15.20	8.90	.80	9.70	63.82
257.....	24.73	12.10	3.25	15.35	62.07
253.....	21.25	12.55	2.90	15.45	72.71
443.....	17.41	11.85	3.35	15.20	87.31
255.....	25.80	16.15	1.65	17.80	68.99
Average.....	19.88	12.28	2.55	14.83	75.32
Average for Groups 1 and 2.....	18.62	10.48	2.31	12.79	70.15

* 3-day average.

Cow 123, a junior 4-year-old registered Guernsey weighing 810 pounds, was slaughtered at 9.30 a. m. on December 2, 1926. She had produced 8,535 pounds of milk and 477 pounds of fat in one year at the age of 2 years, 5 months. She was in the seventh month of her second lactation period. Her udder was unbalanced, the right half being smaller than the left, and the right rear quarter being entirely inactive. For a number of days before death she was led each morning from the barn to the slaughterhouse and back so that the trip on the day of slaughter would not cause undue excitement. Every attempt was made to acquaint her with her new surroundings, but

her udder was not handled nor was she in any way treated to stimulate the letting down of milk either on previous days or on the morning of slaughter. For the 10 days previous to slaughter her production ranged from 9.8 to 11 pounds and averaged 10.37 pounds when she was milked once daily. Her schedule of feeding and watering was kept as nearly uniform as possible. She was killed by a blow on the head and subsequent bleeding. Death was reasonably sudden and without excessive struggle. The temperature of the air in the slaughterhouse was close to freezing on the day of slaughter. Forty minutes elapsed between time of killing and commencement of milking, during which time the udder was amputated and attached to an iron frame.

The time required for daily milking before death was 5 to 7 minutes. The post-mortem milk was drawn more slowly and with somewhat greater difficulty, the first milking requiring 34 minutes, and the second, 10 minutes. The temperature of the milk during the 10 days before death ranged from 34° to 36° C. The temperature of the milk of the first post-mortem milking was approximately 29½° near the beginning and 25° near the finish. The temperature of the milk of the second and final post-mortem milking was approximately 25½°. The temperature of the inside of the udder was not determined. The udder remained in the slaughterhouse for nearly two hours, after which it was removed to a room having a temperature of about 24° to 27°, where it was kept until final milking was complete. Undoubtedly the udder was chilled considerably during the first two hours after its amputation.

Cow 272, a senior 4-year-old registered Holstein weighing 1,200 pounds, was slaughtered at 9.30 a. m. on December 22, 1926. Her best record of production for one year, which was made at the age of 2 years, amounted to 12,762 pounds of milk and 447 pounds of fat. She was in the seventh month of her third lactation period when killed. Her udder was large, well attached, slightly tilted, and slightly quartered, but functioning in all quarters. On December 18 the left front quarter showed an infection and a reduced milk flow. The infected quarter responded to treatment, and on December 20 and 21 appeared again to be normal in condition and in quantity of production. For 10 days before death she was led from the barn to the slaughterhouse and back each day, as in the case of cow 123. During this 10-day period, her production ranged from 20.0 to 24.2 pounds and averaged 21.83 pounds on one milking daily. Her schedule of feeding and watering was kept as nearly constant as possible. She was killed by a blow on the head and subsequent bleeding. Several strokes were required to fell her. She struggled and kicked vigorously, and her muscles apparently were tense at and immediately after death. Considerable importance is attached to the possible effects of failure to obtain sudden death without excessive struggling. The temperature of the air in the slaughterhouse was low. Forty-five minutes elapsed between killing and commencement of milking, during which time the udder was amputated and suspended.

The time required for daily milking before death was 7 to 11 minutes. At each milking on December 18 to 21, inclusive, the cow was milked as before, for 9 to 10 minutes, and the major portion of the milk so obtained was weighed. The udder was then massaged and stripped for 15 minutes more. The massaging and stripping resulted in the recovery of an additional 0.1 to 0.5 of a pound of milk

daily, and for the four days averaged 0.25 of a pound. The post-mortem milking was much slower and more difficult. In the first 9 minutes only 4 pounds of milk had been drawn, and its temperature was approximately $29\frac{1}{2}^{\circ}$ C. At the end of 45 minutes 6.5 pounds of milk had been drawn, and its temperature was about 22° . The second post-mortem milking occupied 25 minutes. The temperature of this milk also was 22° . The temperature of the milk drawn during the 10 days before death had ranged from 36° to 37° . The temperature of the inside of the udder was not determined. The udder remained in the slaughterhouse at a low temperature for about two hours, after which it was sent to a warm room, where it remained until after the second post-mortem milking. Undoubtedly this udder, like the previous one, was decidedly chilled before it was removed to the warm room.

On an average, the first four amputated udders yielded 61.10 per cent as much milk as the cows had produced at corresponding milkings before death. (Table 1.) Those yielding the greatest quantity before death liberated a lower percentage of that quantity after death. The udders of the two cows that struggled violently at the time of slaughter yielded only 49.58 and 40.31 per cent, respectively, whereas the udders of the two cows that died without excessive struggle or noticeable tension of muscles liberated 85.09 and 69.43 per cent, respectively, of the quantities produced at similar milkings before death.

The smaller proportion of milk liberated after death, and the greater difficulty in drawing it from the amputated udders of the two cows that became excited, struggled violently, and died with tense muscles, suggest the possibility that these factors may have been responsible for the difficulty experienced in drawing the milk as readily or as completely as from the others. In this connection, it is suggested that the nervous shock produced by killing the cow and to some extent by severing the nerves in the process of amputating the udder, may result in the development of an abnormal condition in the udder more or less comparable to that produced by pain or fright, which apparently causes the living cow to hold up her milk.

CONTINUATION OF EXPERIMENTS UNDER MORE CAREFULLY CONTROLLED CONDITIONS WITH SEVEN COWS

The necessity for repeating the experiments under more carefully controlled conditions was apparent. Therefore, in the second group of experiments, the results of which are presented in Tables 1 and 2, an attempt was made (1) to prevent as far as possible all excitement of the cow before death, (2) to cause sudden death without excessive struggling, and (3) to maintain approximately blood temperature within the amputated udder until after the completion of post-mortem milking.

As a beginning for this study, three grade Holstein cows were obtained from the dairy herd of St. Elizabeths Hospital in Washington, D. C. They were in average flesh and condition, but were inclined to be high-strung, nervous, and excitable. The cows were placed in box stalls in the building where slaughter was to take place, and were kept as quiet as possible. A regular program of feeding, watering, and milking was adopted. In an effort to obtain sudden death without

excessive struggling, the cows were shot through the brain. In order that the inside of the udder might be held approximately at blood temperature, a wooden box was constructed of sufficient size to hold the iron frame from which the udders were suspended. (Fig. 1.) The box was provided with an electric heater, a glass window in the front for purposes of visibility, and a slide in the end to serve as a ventilator. Below the front window an opening was provided and

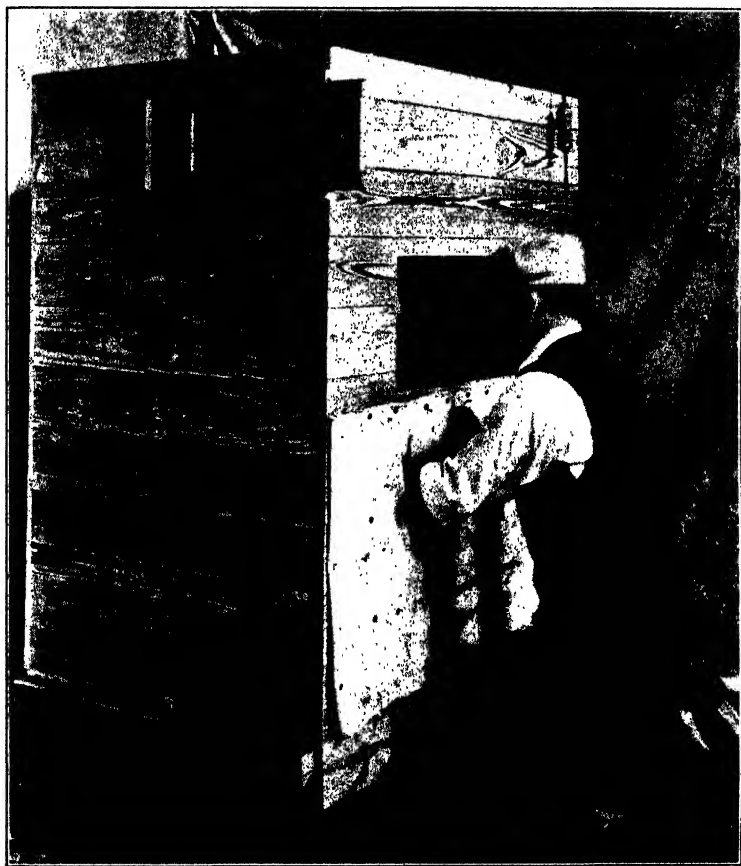


FIGURE 1.—Box for controlling temperature of the udder in post-mortem milking

covered with a heavy canvas. A thermometer was suspended within the box in such a position that readings could be made from the outside.

Immediately after the cow was shot cloths soaked in hot water were wrapped about the udder. Frequently changed hot cloths were kept on the udder during the time required for suspending and bleeding the cow and amputating the udder, attaching it to the frame, and inserting it in the box. From the time the udder was inserted until the final post-mortem milking was completed, the temperature of the

box was kept at about 47° to 48° C. A thermometer, inserted in one of the large blood vessels, permitted a determination of the inside temperature of the gland. Milking was commenced at once. It was accomplished by reaching through slits in the canvas front and milking in the usual manner. The udder was visible to the milker through the window.

Cow 846, a 6-year-old grade Holstein weighing 1,075 pounds, was slaughtered at 9.30 a. m. on April 6, 1927. Her best record for one year was 12,220 pounds of milk made at the age of 4 years 9 months, on two milkings daily. At the time of slaughter she was beginning the fifth month of lactation. Her udder apparently was normal, relatively small, and somewhat tilted but unusually loose, flexible, and mellow, with comparatively little tissue present when milked out. During the 10 days immediately before slaughter her production ranged from 17.4 to 19.7 pounds and averaged 18.51 pounds when she was milked once daily. Shooting through the brain resulted in almost instantaneous death with hardly any struggle or apparent tenseness of muscles. She was bled immediately. Forty-eight minutes was required for killing and bleeding her and for amputating the udder, attaching it to its supporting frame, and placing it in the temperature-control box for milking.

The quantity of milk obtained was 15.4 pounds in the first and 3.3 pounds in the second post-mortem milking, a total of 18.7 pounds or 101.03 per cent of the average daily production for the 10 previous days. Milking of the amputated udder was remarkably easy and rapid. The major portion of the first post-mortem milking was drawn in 6 minutes. Massaging and stripping was continued for 7 minutes. The second post-mortem milking occupied 11 minutes. During the four days immediately before death the time required for milking the major portion varied from 6 to 7 minutes. Subsequent massaging and stripping on these days yielded only 0.3 to 0.4 of a pound daily. The temperature of the milk during the four days before death ranged from 34° to 36° C. The temperature of the major portion of the first post-mortem milking was 35°, whereas at the end of the stripping period it was 33°. The temperature of the second post-mortem milking was 35½° C. Some difficulty was experienced in reading these temperatures because of the position of the milk bucket in the temperature-control box and because of the milk foam which adhered to the thermometer. The temperature of the control box was maintained between 45° and 49° until after completion of the second post-mortem milking. The temperature of the inside of the udder registered between 37° and 38°. The air temperature at the slaughterhouse was 18½° at 2.30 p. m.

Cow 903, a 5-year-old grade Holstein weighing 1,050 pounds, was slaughtered at 9.30 a. m. on April 11, 1927. Her best production for one year was 12,943 pounds of milk, made at the age of 3 years 11 months, on two milkings daily. She was in her fifteenth month of lactation. Her udder was of medium size, fair shape, except for being light in the left rear quarter, very loose, yielding, and mellow, but inferior in quality to that of cow 846. Her milk production for the previous 10 days ranged from 15.3 pounds to 17.7 pounds and averaged 16.27 pounds when she was milked once daily. Death by shooting was practically instantaneous and without struggle. She

was bled immediately. Forty-five minutes elapsed between the time of shooting the cow and commencement of post-mortem milking.

The quantity of milk obtained by hand milking was 9 pounds in the first and 2.6 pounds in the second post-mortem milking, a total of 11.6 pounds, which is 71.3 per cent of the average quantity obtained during the previous 10 days. Post-mortem milking was comparatively easy and rapid, but less so than in the case of cow 846. The major portion of the first post-mortem milking was drawn in 10 minutes, after which the udder was massaged and milked for 9 minutes. The second post-mortem milking occupied 11 minutes. During the nine days before death the time required for drawing the major portion had ranged from 6 to 10 minutes. Subsequent massaging and stripping on these days had yielded only 0.4 to 0.8 of a pound daily. The temperatures of the milk during the nine days before death were 34° and 35° C. The temperature was 35° for the major part and 33° for the strippings of the first, and 34½° for the second post-mortem milking. The temperature in the control box was maintained at 45½° to 48° throughout the period of post-mortem milkings. The temperatures of the inside of the udder during the same period ranged from 36½° to 39°. The outside air temperature was 12° at 9.30 a. m. and 14½° at 2.30 p. m.

Cow 811, a 6-year-old grade Holstein weighing 1,280 pounds, was slaughtered at 9.15 a. m. on April 15, 1927. Her best production for one year was 10,928 pounds of milk, made at the age of 4 years 9 months, on two milkings daily. She was just commencing her twenty-fifth month of lactation. Her udder was medium to small, of fair shape, with light and almost dry left rear quarter and with garget in the left front quarter. The udder was loose and yielding, and contained fibrous tissue of medium softness. In quality it was the poorest of the three in the group. Her milk production during the 10 days previous to slaughter ranged from 11.7 to 17.6 pounds and averaged 15.2 pounds on one milking daily. She was shot through the brain, and death was practically instantaneous and without struggle. Bleeding and amputation of the udder followed in rapid succession. Forty-four minutes elapsed between shooting and commencement of milking.

The quantity of milk obtained by hand milking the amputated udder was 8.9 pounds for the first and 0.8 pound for the second post-mortem milking, a total of 9.7 pounds or 63.82 per cent of the average quantity yielded during the previous 10 days. The milking was fairly easy and rapid but not so readily accomplished as in the case of cow 846. Seven minutes was required for drawing the major portion, and 6 minutes for massaging the udder and drawing the remainder of the first post-mortem milk. The second post-mortem milking required 10 minutes. During the previous 10 days the time required for drawing the major portion had ranged from 6 to 10 minutes and the temperature of the milk had ranged from 34° to 36° C. On these days subsequent massaging and stripping had yielded 0.2 to 0.5 of a pound. The temperature of the milk was 37° for the major portion and 34½° for the strippings of the first post-mortem, and 33° for the second post-mortem milking. In this case a third post-mortem milking was attempted approximately two hours after the second, for the purpose of determining whether

additional milk could be obtained in significant quantities. Six minutes of massaging and stripping yielded only approximately 0.05 of a pound. The temperature of the control box was maintained between 45° and 50° until the completion of the third post-mortem milking except that immediately after the udder was inserted, the temperature reading was 38°. The temperature of the inside of the udder during this period ranged from 37° to 39°. Outside air temperatures on this day were 12½° at 9.30 a. m. and 17° at 2 p. m.

From time to time lactating cows in the herd of the Bureau of Dairy Industry at Beltsville, Md., became available for similar experiments. The work performed with each of four additional cows, making a total of seven in the second group, is discussed separately.

Cow 257, a 10-year-old registered Holstein, was slaughtered at 9.15 a. m. on February 21, 1928. She weighed 1,194 pounds, which was considerably below her usual mature weight. Her best production record for one year was 24,135 pounds of milk and 852 pounds of fat, made at 6 years 4 months of age on three milkings daily. She was in the second month of lactation when slaughtered. Her udder was somewhat unbalanced, of good size, fair shape, and very loose, and contained tissue that was inclined to be hard and fibrous. She had been kept on a regular schedule of feeding and watering and was milked twice daily throughout a 10-day period. The morning milking commenced at 9.15 a. m. Production at the morning milking during this 10-day preliminary period ranged from 21.6 to 27.7 pounds and averaged 24.73 pounds. Death, which resulted from shooting through the brain, was almost instantaneous and was accompanied with very little struggling or tenseness of muscles. Bleeding of the animal and amputation of the udder followed in rapid succession. Sixty-three minutes elapsed between shooting and commencement of milking.

Hand milking of the amputated udder yielded 12.10 pounds in the first and 3.25 pounds in the second post-mortem milking, a total of 15.35 pounds or 62.07 per cent of the average quantity obtained during the previous 10 days. In order to make possible a subsequent study, the two halves of the udder were milked separately in both the first and second post-mortem milkings. The time required for drawing the milk, therefore, was somewhat greater than otherwise would have been necessary. Ten minutes was required for drawing the major portion in the first post-mortem milking, after which the udder was massaged and stripped. The second post-mortem milking required 17 minutes. The time for drawing the major portion of the milk at corresponding milkings during the preliminary period, when both halves were milked simultaneously, ranged from 8 to 12 minutes and averaged 10 minutes, and the temperature of the milk, which was taken on the last six days, ranged from 35° to 38° C. During the 10-day period, subsequent massaging and stripping yielded from 0.5 to 1 pound and averaged 0.8 of a pound of milk. The temperature was 32½° for the major portion and 22° and 26½° for the two separate parts of the strippings of the first post-mortem milking. The temperature of the second post-mortem milk was 27½° for each part.

The temperature within the control box ranged from 45° to 52° C., and the temperature of the inside of the udder ranged from 36° to 38°. The air temperature in the slaughter room was approximately

1½° at the time of slaughter and 4½° at the end of the second post-mortem milking.

Cow 253, a 9-year-old registered Holstein weighing 1,263 pounds, was slaughtered at 9.19 a. m. on June 11, 1928. Her best production record for one year was 16,405 pounds of milk and 628 pounds of fat, made at 5 years 8 months of age on three milkings daily. She had been lactating 14 months at the time of slaughter. Her udder was of medium to good size, medium shape, and excellent flexibility, but the tissue within was medium to hard and very fibrous. She had been fed and watered regularly and milked once daily at 9.15 a. m. for 10 days, during which her production ranged from 19.3 to 22.5 pounds and averaged 21.25 pounds daily. The first shot from a 22-caliber rifle was not effective and the cow became violent. A second shot was effective, but her muscles became extremely tense. Bleeding and amputation of the udder followed as quickly as possible, but 64 minutes elapsed between the time of shooting and the commencement of milking of the amputated udder.

Hand milking yielded 12.55 pounds in the first and 2.90 pounds in the second post-mortem milking, a total of 15.45 pounds or 72.71 per cent of the average quantity obtained at corresponding milkings during the previous 10 days. Eight minutes was required for drawing the major portion of the first post-mortem milk, after which the udder was massaged and stripped for 7 minutes. The second post-mortem milking required 8 minutes. During the previous 10 days the drawing of the major portion had required 8 to 9 minutes and averaged 8½ minutes, and the temperature of the milk ranged from 37° to 38° C. Subsequent massaging and stripping during the 10-day period yielded an average of 0.71 of a pound. The temperature of the milk of the first post-mortem milking was 34° for the major portion and 29½° at the end of stripping; that of the second post-mortem milking was 32°.

The temperature of the control box ranged from 45° to 52° C. The temperature of the inside of the udder was determined after the first, and again after the second post-mortem milking, and in each case was 38°. The air temperature was 17½° at the time of slaughter and 17° at the completion of the second post-mortem milking.

Cow 443, a 9-year-old registered Jersey weighing 855 pounds, was slaughtered at 9.30 a. m. on June 12, 1928. Her best record of production for one year was 9,621 pounds of milk and 465 pounds of fat, made at 6 years 4 months of age on three milkings daily. She was in the seventh month of lactation when slaughtered. Her udder was large, of good shape, and very loose and yielding, but contained fibrous tissue of more than average mellowness. She had been kept on a regular schedule of feeding and watering and had been milked regularly once daily at 9.30 a. m. through a 10-day period, during which her milk production ranged from 14.6 to 19.5 pounds and averaged 17.41 pounds daily. Death from shooting was almost instantaneous and was accompanied by little struggling, although the muscles became relatively tense. Bleeding and amputation of the udder followed in rapid succession and within 59 minutes from the time of shooting, the udder was placed in the temperature control box and milking was commenced.

Hand milking of the amputated udder yielded 11.85 pounds in the first and 3.35 pounds in the second post-mortem milking, a total of 15.20 pounds or 87.31 per cent of the average quantity obtained in corresponding milkings during the previous 10 days. Twelve minutes was required for drawing the major portion of the first post-mortem milk, after which the udder was massaged and stripped for four minutes. The second post-mortem milking required eight minutes. During the previous 10 days, the drawing of the major portion had required from 7 to 9 minutes and averaged 7.9 minutes, and the temperature of the milk ranged from 37° to 38° C. Subsequent massaging and stripping on these 10 days yielded an average of 0.72 of a pound. The temperature of the milk obtained at the first post-mortem milking was 34½° for the major portion and 31° at the end of stripping. The temperature of the milk obtained at the second post-mortem milking was 32°. The temperature of the control box ranged from 40° to 60°. This wide variation was due to breakage of the heating coil. The temperature of the udder tissue after the second post-mortem milking was 37°. The air temperature was 19½° at the time of slaughter and 22° at the time of the second post-mortem milking.

Cow 255, a 9-year-old registered Holstein weighing 1,462 pounds, was slaughtered at 9.05 a. m. on February 1, 1929. Her best record of production for one year was 11,202 pounds of milk and 415 pounds of butterfat, made at 2 years 8 months of age on three milkings daily. She was slaughtered 32 days after the last freshening. She had been kept on a regular schedule of feeding and watering, and had been milked once daily at 9 a. m. for 10 days, during which her milk production ranged from 23.1 to 29.2 pounds and averaged 25.8 pounds daily. Her udder was of medium size, poor shape, very loose and mellow, and contained a medium quantity of mellow, fibrous tissue which was inclined to be stringy. Death from shooting was almost instantaneous and was accompanied by a minimum of struggling or tenseness of muscles. Bleeding and amputation of the udder followed, and 79 minutes after the cow was shot, milking in the temperature control box was commenced.

Hand milking yielded 16.15 pounds in the first and 1.65 pounds in the second post-mortem milking, a total of 17.80 pounds or 68.99 per cent of the average quantity obtained at corresponding milkings during the previous 10 days. The milk was drawn easily and rapidly. Eleven and one-half minutes was required for drawing the major portion of the first post-mortem milk, after which the udder was massaged and stripped for an equal period of time. Massaging and stripping yielded only 1.1 pounds of milk. The second post-mortem milking required 11 minutes. During the previous 10 days the drawing of the major portion of the milk had required from 9 to 11 minutes and averaged 10.2 minutes, and the temperature of the milk on the last 8 of these days had ranged from 34° to 36° C. Subsequent massaging and stripping during this 10-day period yielded an average of 1.05 pounds. The temperature of the milk obtained at the first post-mortem milking was 33° for the major portion and 31½° at the end of stripping; that of the second post-mortem milk was 30°. The temperature of the control box ranged from 22½° when the udder was inserted to 50° at the time of final milking. The low temperature at the start resulted from an attempt to generate sufficient heat with

a small electric plate when the air temperature was -1° in the slaughter room. Soon after the udder was inserted a different heating unit was used and the results were more satisfactory. The inside temperature of the udder at the end of the first post-mortem milking was $34\frac{1}{2}^{\circ}$. This temperature increased steadily, reaching $38\frac{1}{2}^{\circ}$ at the time of the second post-mortem milking. The air temperature in the slaughter room was 3° at 3.30 p. m.

COMPARISON OF RESULTS OBTAINED WITH THE TWO GROUPS OF COWS

In the first group of cows studied an average of 61.10 per cent as much milk was obtained from the amputated udders as from the same udders at corresponding milkings before death of the cows. The minimum post-mortem recovery for cows of this group was 40.31 per cent, and the maximum was 85.09 per cent. In the second group the average post-mortem recovery was 75.32 per cent, with a minimum of 62.07 and a maximum of 101.03 per cent. The average for both groups was 70.15 per cent. These values are obtained by dividing the sum of the percentages in a group by the number of cows in the group. Such a method is permissible, as the work with each cow is considered as an independent experiment having equal importance with every other one.

The average producing level for each cow was 3.47 pounds greater in the second group than in the first, and the quantity of milk obtained from the amputated udders was 5.61 pounds greater in the second group than in the first. If it were true that the milk in the udder at the beginning of milking represents only a small proportion of the quantity obtained at that milking, one would expect a greater percentage of recovery from those amputated udders that had yielded the smaller quantities before death. Such, however, was not the case, as the amputated udders of the second or higher-producing group of cows yielded not only a greater quantity but a greater proportion, as shown by the average post-mortem recoveries of 61.10 and 75.32 per cent, respectively, for groups 1 and 2. Of the total quantity of milk recovered from the amputated udders, 81.94 per cent was obtained at the first post-mortem milking and only 18.06 per cent at the second.

The percentages in Table 1 are, with one exception, based on the average production of each cow at corresponding milkings on the 10 days immediately preceding slaughter. In general the production of milk during the preliminary 10-day period before slaughter was reasonably uniform, but in a few instances production was increasing, which tends to make the percentage showing post-mortem recovery higher than it would have been if only the last two days before slaughter had been used as a basis for comparison. In other instances the cow's production was decreasing, and this resulted in the percentage showing post-mortem recovery being somewhat smaller than it would have been if the comparisons had been made on a 2-day basis.

The post-mortem recovery has been computed also on the basis of the yield for the two days immediately preceding slaughter. (Table 2.) The results are essentially the same whether the percentage of post-mortem recovery of milk from amputated udders is based on the milking level for the 10 days or for the two days preceding slaughter.

TABLE 2.—*Relative post-mortem recoveries of milk from amputated udders, calculated on the basis of the production for the previous 10-day and 2-day periods*

Group and number of cows	Percentage of average quantity obtained on previous 10-day period	Percentage of average quantity obtained on previous 2-day period
Group 1, 4 cows.....	61.10	61.10
Group 2, 7 cows.....	75.32	75.52
Groups 1 and 2 combined, 11 cows.....	70.15	70.27

DISCUSSION

The average recovery of milk obtained by milking the 11 amputated udders (70.15 per cent) was a much greater proportion of the ante-mortem yield than that indicated by the experiments of Gaines and Sanmann. (1)

In contrast to the method of milking one half of the udder before and the other half after slaughter, which was employed by Petersen and his associates, (6) the writers carefully avoided touching the udder or in any other way stimulating the giving down of the milk immediately before slaughter, except in the case of one of the first cows whose udder was examined for quality of tissue.

Inasmuch as the changes in the manner of killing the cow and in the system of controlling the temperature of the udder after death were both introduced at the same time, the specific effect of each on the quantity of milk recovered from the amputated udders could not definitely be determined. Because of the brevity of the period between killing the cow and commencing the first post-mortem milking, however, it would appear that controlling the temperatures could have had little opportunity to affect the quantity of milk recovered at that milking, and that the greater recovery from amputated udders in Group 2 as compared with that in Group 1 was due chiefly to the effect of changing the method of slaughter. It seems reasonable to assume, however, that during the 4-hour interval between the first and second post-mortem milkings the effect of temperature might have been considerably greater than that of the method of killing on the total quantity of milk obtained.

The high average recovery of milk from amputated udders (61.10 per cent for Group 1, 75.32 per cent for Group 2, and 70.15 per cent for Groups 1 and 2 combined), together with indications that in some cases milk remained in the udders after the second post-mortem milking, which, considering the shock and abnormal conditions incidental to slaughter, is not surprising, offer almost conclusive evidence that practically all the milk obtained at a milking is present in the udder when milking is commenced. Reference to the possibility that the shock of slaughtering may have been partly responsible for the residue of milk observed in some of the udders after completion of the second post-mortem milking suggests the possibility of some mechanism which enables a cow to hold up her milk. This ability of some cows to withhold their milk under certain circumstances is well known. The nature of such a mechanism is not known, but many theories have

been advanced, of which the one expressed by Klein (4, p. 8-9) is particularly interesting. From the beginning of the study of this subject the writers' observations have indicated that milk secretion is a continuous process, except when it is interrupted or retarded by such factors as sickness, injury, or pressure in the udder resulting from accumulation of milk. This latter fact also explains why heavy-producing cows yield more milk when milked three or four times daily than when milked only twice daily. The removal of the milk at shorter intervals prevents the development of sufficient pressure to check secretion and permits it to proceed at a uniform rate. It also is in accord with the common practice of drying off cows by allowing the milk to accumulate in the udder. In the drying-off process the pressure developed is evidently sufficient to stop secretion completely until the secreting cells cease to function.

Since the milk is already stored in the udder when milking is commenced, the importance of an adequate size and capacity of udder is obvious.

It has been more or less generally assumed that if a residue of milk is left in the udder at milking time the production of the cow will be diminished if not completely arrested. The necessity for completeness of milking may be questioned, for even though the cow is thoroughly milked at milking time the udder will again contain milk in a very short time.

The fact that a number of investigators using different methods have reported data which lead to almost the same conclusions regarding the continuous secretion and storage of milk in the udder greatly strengthens the deductions outlined in a previous report (9), as well as those here presented.

SUMMARY

This paper discusses the individual performance of 11 cows arranged in two groups. The cows in Group 1 were felled by a blow on the head and their udders were permitted to become chilled after death. The cows in Group 2 were killed by shooting and their udders were held at approximately blood temperature until after the second post-mortem milking was completed.

The average recovery of milk from the amputated udders was 61.10 per cent of the ante-mortem yield for Group 1, 75.32 per cent for Group 2, and 70.15 per cent for Groups 1 and 2 combined.

Approximately 80 per cent of the total amount of milk recovered from the amputated udders was obtained at the first and approximately 20 per cent at the second post-mortem milking. Exact figures showing the relation between the quantities obtained at the first and second post-mortem milkings are 79.50 per cent and 20.50 per cent for Group 1, 82.81 per cent and 17.19 per cent for Group 2, and 81.94 per cent and 18.06 per cent for Groups 1 and 2 combined. The relation between the recoveries of milk in the first and second post-mortem milkings is, therefore, almost the same in Group 2 as in Group 1.

LITERATURE CITED

- (1) GAINES, W. L., and SANMANN, F. P.
1927. THE QUANTITY OF MILK PRESENT IN THE UDDER OF THE COW AT MILKING TIME. *Amer. Jour. Physiol.* 80: 691-701.
- (2) GOWEN, J. W., and TOBEY, E. R.
1927. UDDER SIZE IN RELATION TO MILK SECRETION. *Jour. Gen. Physiol.* 10: 949-960, illus.
- (3) ——— and TOBEY, E. R.
1928. SIGNIFICANCE OF THE CHEMICAL COMPOSITION OF THE SECRETING AND DRY MAMMARY GLAND TO MILK SECRETION. *Jour. Gen. Physiol.* 12: 123-128.
- (4) KLEIN, L. A.
1917. PRINCIPLES AND PRACTICE OF MILK HYGIENE. 329 p., illus. Philadelphia and London.
- (5) PETERSEN, W. E.
1928. SOME ASPECTS OF THE PHYSIOLOGY OF MILK SECRETION WITH SPECIAL REFERENCE TO MILK FAT. *Amer. Assoc. Med. Milk Comms. (Inc.), and Certified Milk Producer's Assoc. of America (Inc.), Proc. Ann. Conf.* 22: 134-144.
- (6) ——— PALMER, L. S. and ECKLES, C. H.
1929. THE SYNTHESIS AND SECRETION OF MILK FAT. THE TIME OF MILK AND FAT SECRETION. *Amer. Jour. Physiol.* 90: 573-581.
- (7) RAGSDALE, A. C., ELTING, E. C., GIFFORD, W., and BRODY, S.
1927. STUDIES IN MILK SECRETION: (A) TIME RELATIONS IN MILK SECRETION; (B) MECHANISMS REGULATING VARIATIONS IN THE COMPOSITION OF MILK. *Missouri Agr. Expt. Sta. Bul.* 256: 63-64.
- (8) ——— TURNER, C. W., BRODY, S., ELTING, E. C., and GIFFORD, W.
1926. STUDIES IN MILK SECRETION: (A) TIME RELATIONS IN MILK SECRETION; (B) MECHANISMS REGULATING VARIATIONS IN THE COMPOSITION OF MILK. *Missouri Agr. Expt. Sta. Bul.* 244: 32.
- (9) SWETT, W. W.
1927. RELATION OF CONFORMATION AND ANATOMY OF THE DAIRY COW TO HER MILK AND BUTTERFAT PRODUCING CAPACITY. UDDER CAPACITY AND MILK SECRETION. *Jour. Dairy Sci.* 10: 1-14.

COMPOSITION OF MILK OBTAINED FROM AMPUTATED COW UDDERS¹

By W. W. SWETT, *Senior Dairy Husbandman*; FRED W. MILLER, *Senior Veterinarian and Physiologist*; and R. R. GRAVES, *Chief, Division of Dairy Cattle Breeding, Feeding, and Management, Bureau of Dairy Industry, United States Department of Agriculture*²

INTRODUCTION

In an early report (7)³ of the milk secretion studies which have been in progress for several years in the Bureau of Dairy Industry, United States Department of Agriculture, the quantity and composition of the milk obtained from two amputated cow udders was compared with that obtained from the same udders at corresponding milkings before the death of the cow. Another paper (8), dealing with quantity of milk, gave a similar comparison of the results obtained with 11 cow udders and showed that most, if not all, of the milk obtained at a milking is present in the udder when milking is commenced. The results given also indicated that milk secretion is a continuous process except when it is interrupted or slowed down by such factors as sickness, injury, or pressure in the udder resulting from the accumulation of milk.

The milk obtained from the two amputated udders (7), as compared with that obtained at the last two corresponding ante-mortem milkings, differed more in butterfat than in any other constituent. In the milk drawn at the first post-mortem milking, which took place as soon as possible after amputation of the udder, and in the milk of the second post-mortem milking, performed four hours later, the butterfat test averaged approximately 61 and 26 per cent, respectively, as high as it did in the milk obtained at the last two corresponding ante-mortem milkings. This reduction in butterfat in the milk obtained after death was attributed to the fact that the amputated udders were allowed to become chilled and that, as a result, some of the butterfat may have solidified and adhered to the lining of the ducts.

The percentage of total solids in the milk obtained from the first two cows (7) showed a steady decline from the ante-mortem to the first post-mortem and the second post-mortem milkings. The percentage of solids not fat was slightly higher in the milk from the first post-mortem milking than in the ante-mortem milk, but it was lower in the milk from the second post-mortem milking than in either the ante-mortem or the first post-mortem milk. The percentage of ash increased steadily from the ante-mortem to the second post-mortem milking. The percentage of total protein was less consistent but showed a very slight upward trend from the ante-mortem to the first post-mortem milking, and a somewhat greater downward trend from the first to the second post-mortem milking, with the second post-mortem slightly lower in percentage of total protein than the ante-

¹ Received for publication, Feb. 4, 1932; issued October, 1932.

² Acknowledgment is made to L. A. Rogers and his associates in the research laboratories, Bureau of Dairy Industry, for analyses of milk samples.

³ Reference is made by number (italic) to Literature Cited, p. 418.

mortem milking. The percentage of lactose showed a moderate upward trend from ante-mortem to first post-mortem and a sharp decline from first to second post-mortem milking.

DISCUSSION OF WORK OF OTHER INVESTIGATORS

Gaines and Sanmann (1) concluded that at the time of milking there is present in the udder of the cow a quantity of milk in excess of that obtained upon milking. This conclusion was based on the fact that they were able to recover a greater quantity of lactose from the hashed, amputated udder and its contents than from the milk obtained from the same udders at corresponding ante-mortem milkings. Similarly, Gowen and Tobey (2) were able to recover from 16 of 20 udders studied, more than enough lactose to account for the quantity of milk obtained at corresponding milkings before death. The conclusions of Gowen and Tobey appear to be essentially the same as those of Gaines and Sanmann. The conclusions drawn by both of these groups of investigators, however, seem to be based on the assumption that the lactose content of milk is stable and that the recovery of lactose from the amputated udder indicates the presence of milk having the same lactose content. In a later publication Gowen and Tobey (3) showed that, at the time of milking, there are also sufficient quantities of fat, ash, and nitrogen to more than account for all the milk produced. Their earlier deductions are greatly strengthened by evidence that the nonlactating udder does not contain any lactose.

Ragsdale and his associates (6) working with two amputated udders, obtained 36.50 per cent and 57.96 per cent of the quantity of milk obtained at corresponding ante-mortem milkings, but only 15.90 per cent and 46.44 per cent, respectively, of the normal amount of butterfat. This would indicate that in the first case the butterfat test of the post-mortem milk was only 43 per cent as high, and in the second case only 80 per cent as high as that of the ante-mortem milk; an average for the two of slightly more than 60 per cent.

Petersen (4, p. 143) reported that "post-mortem milks are normal in all respects except their fat content, which is low." Petersen, Palmer, and Eckles (5, p. 579) reported that "in every case the fat percentage and total yield of fat in the post-mortem milkings are noticeably smaller than in the in-vivo milkings." They also made the interesting observation that when the post-mortem milking was sampled as the milking progressed, the fat percentage rose to a maximum and then dropped almost to the level of the first portion. The weighted average tests for the six cows included in their study were 4.83 per cent for the ante-mortem milking of the left gland, and 3.12 per cent for the post-mortem milking of the left gland. The average test of the post-mortem milk from the left half of the six udders was, therefore, 64 per cent as high as the test of the ante-mortem milk from the left half of the same udders. Their post-mortem recovery of 51.8 per cent as much total butterfat in the left half of the six udders as in the left half of the same udders at corresponding ante-mortem milkings, and their 80.3 per cent recovery of milk on the same basis, also indicate a test 64 per cent as high in the post-mortem as in the ante-mortem milk. It is interesting that the relation of the post-mortem to ante-mortem test observed by Petersen, Palmer, and

Eckles is almost the same as that reported by Ragsdale and his associates.

In contrast to all of the results discussed, are those obtained by Olson and Copeland.⁴ Their average butterfat tests of ante-mortem milk, first post-mortem milk, and second post-mortem milk or stripplings were 3.73, 3.55, and 6.48 per cent, respectively. In their work the first post-mortem milk tested nearly as high in butterfat as did the ante-mortem milk, and the second post-mortem milk tested nearly twice as high as the first post-mortem milk.

CONDUCT OF EXPERIMENT

As shown in a previous paper (8), the cows used in these experiments were put on a uniform schedule of feeding, watering, and milking, which, except in one case, was continued for 10 days. During this preliminary period the lactating level of each cow was determined. The composition of the ante-mortem milk was determined from samples taken on the two days immediately preceding slaughter.

As previously reported (8), the 11 cows used in these tests were divided into two groups. Group 1 includes the first four cows studied. These were killed by a blow on the head, and they became more or less violent at the time of death. Their udders were allowed to remain in the abattoir and become chilled before the post-mortem milking was completed. Group 2 includes seven cows which were studied after the program of control had been better standardized. Each of the seven was killed by shooting, and in nearly every instance death was almost instantaneous. The udders of the seven cows in Group 2 were kept in a control box at approximately blood temperature until after the post-mortem milking was finished.

The quantity of milk obtained in post-mortem milkings of the amputated udders of 11 cows was shown in Table 1 of a previous paper (8) to average 70.15 per cent of the quantity obtained at corresponding milkings before death. The average recovery for the first four cows was 61.1 per cent, but the average recovery for the next seven cows, kept and slaughtered under more carefully controlled conditions, was 75.32 per cent of the ante-mortem yield. The report explains that the cows were slaughtered at the exact hour of the day on which the ante-mortem milking had taken place, and that the milk was drawn in 2 separate milkings, 1 as soon as possible after the amputation of the udder, and 1 four hours later.

Samples of the milk obtained at milkings 48 and 24 hours before slaughter, as well as of the milk obtained at both post-mortem milkings, were taken for analysis. The percentages of total solids, ash, and total protein were determined by chemical analysis; the percentage of butterfat was determined by the Babcock method; and the percentages of solids not fat and of lactose were calculated from the analyses obtained.

The average composition of the milk obtained during the last two days before slaughter was used as a basis for comparing the composition of the milk from the first and second milkings of the amputated udders. The quantity of milk obtained on the last two days before slaughter varied only slightly for each cow. Therefore, the average percentage of any constituent in the two ante-mortem samples from

⁴ OLSON, T. M., and COPELAND, L. Unpublished data on file at the South Dakota Agricultural Experiment Station.

any cow can be determined with sufficient accuracy by adding the percentages for the two samples and dividing the total by two. The averages which appear in column 2 of Tables 1 to 5, inclusive and Table 7, were obtained in this manner. The relative percentages given for each cow in the fifth and sixth columns of these tables were obtained by dividing the figures in columns 3 and 4, respectively, by the corresponding figures in column 2. The average percentages for Group 1, Group 2, and Groups 1 and 2 combined were obtained by adding the percentages for all cows in the group and dividing each total by the number of cows in the group.

COMPOSITION OF MILK OBTAINED FROM AMPUTATED UDDERS AS COMPARED WITH THAT OBTAINED AT CORRESPONDING ANTE-MORTEM MILKINGS

TOTAL SOLIDS

Table 1 gives the percentages of total solids in the milk obtained before slaughter and in that obtained from the amputated udders. In the results for every cow in Group 1, the percentage of total solids in the milk obtained at the first post-mortem milking was lower than that in the ante-mortem milk, and was still lower in the second post-mortem milk than in the first post-mortem milk. With one exception, the results for the cows in Group 2 showed a similar tendency, although the decline from the first to the second post-mortem milking was considerably less marked. The exception noted was so noticeably out of line with the other results that it was undoubtedly an error. The data for cow 253 were, therefore, omitted from the averages for Group 2 and for Groups 1 and 2 combined. Immediately beneath these averages are given the corresponding ones which include all the data for this cow. The percentage of total solids in the milk was in every instance distinctly lower for the first post-mortem milk than for the ante-mortem milk, and with one exception was still lower for the second post-mortem than for the first post-mortem milk.

TABLE 1.—Percentages of total solids in milk obtained from the same udders before death of the cow and after death and amputation of the udder

Group and cow No.	Ante-mortem milkings (average for 2 days)	First post-mortem milking	Second post-mortem milking	First post-mortem value as percentage of ante-mortem value	Second post-mortem value as percentage of ante-mortem value
Group 1:					
450	14.68	13.88	10.23	94.55	69.69
292	10.83	9.61	7.88	88.73	72.76
123	14.23	12.37	10.20	86.93	71.68
272	12.86	10.85	9.19	84.37	71.46
Average	13.15	11.68	9.38	88.65	71.40
Group 2:					
846	13.26	11.69	11.00	88.16	82.96
903	13.25	10.74	10.12	81.06	76.38
811	12.38	10.43	9.73	84.25	78.59
257	10.69	8.71	7.79	81.48	72.87
253	12.14	10.52	* 14.35	86.66	* 118.20
443	13.17	11.25	11.23	85.42	85.27
255	10.83	9.46	9.37	87.35	86.52
Average	{ • 12.26 • 12.25	{ 10.38 10.40	{ 9.87 10.51	{ 84.62 84.91	{ 80.43 85.83
Average for Groups 1 and 2	{ • 12.62 • 12.57	{ 10.60 10.56	{ 9.67 10.10	{ 86.23 86.27	{ 76.92 80.58

* Apparently an error.

* Data for cow 253 excluded.

* Data for cow 253 included.

BUTTERFAT

The butterfat percentages are presented in Table 2. The milk from every cow in Group 1 showed a lower percentage of butterfat in the first post-mortem milk, and a still lower percentage in the second post-mortem milk, than in the ante-mortem milk. The butterfat percentages for the milk obtained at the first and second post-mortem milkings averaged only 57.29 and 27.45 per cent as high, respectively, as that of the ante-mortem milkings. The milk from the second post-mortem milking averaged only 47.9 per cent as high in percentage of butterfat as the milk obtained at the first post-mortem milking. In other words, the percentage of butterfat was reduced almost one-half in the first, and again almost one-half in the second, post-mortem milk. The reduction from ante-mortem to second post-mortem milk was fairly uniform for the four cows in the group. The fact that no effort was made to maintain body temperature within the amputated udders strengthens the assumption that a lowering of the udder temperature might have been to some extent responsible for the decrease in percentage of butterfat in the milk obtained from the amputated udders, particularly the decrease from the first to the second post-mortem milking.

TABLE 2.—Percentages of butterfat in milk obtained from the same udders before death of the cow and after death and amputation of the udder

Group and cow No.	Ante-mortem milkings (average for 2 days)	First post-mortem milking	Second post-mortem milking	First post-mortem value as percentage of ante-mortem value	Second post-mortem value as percentage of ante-mortem value
Group 1:					
459	5.74	4.50	1.60	78.40	27.87
292	2.93	1.28	.70	43.69	23.89
123	5.43	3.20	1.80	58.93	33.15
272	3.74	1.80	.93	48.13	24.87
Average	4.46	2.70	1.26	57.29	27.45
Group 2:					
846	4.08	2.70	2.93	66.18	71.81
903	4.08	1.93	2.20	47.30	53.92
811	3.70	1.80	1.73	48.65	46.76
257	2.83	1.24	1.33	43.82	47.00
253	3.64	2.10	1.70	57.69	46.70
443	4.24	2.00	2.83	47.17	66.75
255	3.34	1.73	2.60	51.80	77.84
Average	3.70	1.93	2.19	51.80	58.68
Average for Groups 1 and 2	3.98	2.21	1.85	53.80	47.32

The amputated udders of the cows in Group 2 were all kept in the temperature-control box until after the second post-mortem milking was finished. The results for this group differ considerably from those for Group 1. In every instance the percentage of butterfat in the first post-mortem milk was lower than that in the ante-mortem milk, averaging 51.80 per cent as high as that in the ante-mortem milk. To this extent the results were similar to those for Group 1, as the percentage of butterfat was reduced almost one-half. The relation between the butterfat percentages in the first and second post-mortem milks for Group 2, however, was very different from

that shown for Group 1. The milk from five of the seven cows in Group 2 showed a higher percentage of butterfat in the second post-mortem than in the first post-mortem milking. For the other two cows the reverse was true. Averages for Group 2 show that the butterfat test⁵ was 51.80 per cent as high in the first, and 58.68 per cent as high in the second post-mortem milk as in the ante-mortem milk. In other words, the decrease in the percentage of butterfat between the ante-mortem milk and the first post-mortem milk obtained from the cows in Group 2 was similar to the corresponding decrease shown for the cows in Group 1, but the second post-mortem milk from the cows in Group 2 showed a higher percentage of butterfat than the first post-mortem milk from the cows in that group.

As previously stated, the chilling of the amputated udders of the cows in Group 1 appears to have been partly, if not entirely, responsible for the reduction in the butterfat test between the first and second post-mortem milkings, since this reduction did not occur when the udders were kept warm. The tendency for a reduction in the butterfat test between the ante-mortem and the first post-mortem milk, however, was not overcome or materially influenced by controlling the temperature of the udder after its amputation.

Since the decreases in percentage of butterfat in the post-mortem milk obtained from udders in Group 2 were distinctly different from those obtained in Group 1, and since these differences in the two series can be accounted for by differences in the temperature at which the udders were held after amputation, the combined averages for Groups 1 and 2 in Table 3 may not be particularly significant. The results for Group 2, however, were obtained under controlled conditions, are reasonably consistent, and should be significant. These results, together with the combined results in both groups, indicate that the butterfat test of milk obtained from amputated udders is only about half as high as that of the milk obtained from the same udders before death.

SOLIDS NOT FAT

In general, the percentages of solids not fat in the milk obtained from cows in Group 1 were higher in the first post-mortem milk than in the ante-mortem milk. (Table 3.) This was true in three of the four cases studied. In each of the four the percentage of solids not fat in the milk from the second post-mortem milking was lower than in the milk of either the ante-mortem or the first post-mortem milking. On an average, the percentages of solids not fat in the milk obtained at the first and second post-mortem milkings were 103.45 and 93.36 per cent, respectively, of the percentages found in the ante-mortem milk.

⁵ "Butterfat test" and "percentage of butterfat" are used interchangeably.

TABLE 3.—Percentages of solids not fat in milk obtained from the same udders before death of the cow and after death and amputation of the udder

Group and cow No.	Ante-mortem milkings (average for 2 days)	First post-mortem milking	Second post-mortem milking	First post-mortem value as percentage of ante-mortem value	Second post-mortem value as percentage of ante-mortem value
Group 1:					
459.....	8.94	9.38	8.63	104.92	96.53
292.....	7.90	8.33	7.18	105.44	90.89
123.....	8.80	9.17	8.40	104.20	95.45
272.....	9.12	9.05	8.26	99.23	90.57
Average.....	8.69	8.98	8.12	103.45	93.36
Group 2:					
846.....	9.18	8.99	8.07	97.93	87.91
903.....	9.17	8.81	7.92	96.07	86.37
811.....	8.68	8.63	8.00	99.42	92.17
257.....	7.86	7.47	6.46	95.04	82.19
253.....	8.50	8.42	• 12.65	99.06	• 148.82
443.....	8.93	9.25	8.40	103.58	94.06
255.....	7.49	7.73	6.77	103.20	90.39
Average.....	{ • 8.55 • 8.54	{ 8.48 8.47	{ 7.60 8.32	{ 99.21 99.19	{ 88.85 97.42
Average for Groups 1 and 2.....	{ • 8.61 • 8.60	{ 8.68 8.66	{ 7.81 8.25	{ 100.90 100.74	{ 90.65 95.94

• Apparently an error.

• Data for cow 253 excluded.

• Data for cow 253 included.

For Group 2, however, the results were slightly different. The first post-mortem milk averaged almost the same as the ante-mortem milk in percentage of solids not fat. For five of the cows the percentages of solids not fat were slightly lower and for two they were slightly higher in the first post-mortem than in the ante-mortem milk. The percentages of solids not fat in this group also were decidedly lower in the second post-mortem than in either the ante-mortem or the first post-mortem milk. This was true in six of the seven cases. The only exception in this group was in the case of cow 253, for which the discrepancy in total solids already has been noted. Since the percentages of solids not fat were determined by subtracting the percentages of butterfat from the percentages of total solids, any outstanding discrepancy in the latter would be correspondingly great in the percentages of solids not fat. The averages for solids not fat have, therefore, been treated the same as in the case of the total solids. (Table 1.) The averages which include the data for cow 253 are given in Table 3 immediately beneath the other averages.

The downward trends in percentage of solids not fat from the first post-mortem to the second post-mortem milk in Groups 1 and 2 are similar. On an average, the actual percentage of solids not fat in the second post-mortem milk was 0.86 per cent lower than that of the first post-mortem milk for Group 1, and 0.88 per cent lower for Group 2. The average figures showing the relation between the solids not fat in the post-mortem and ante-mortem milk are both lower for Group 2 than for Group 1. In general, the first post-mortem milk had almost the same percentage of solids not fat as the ante-mortem milk, but the second post-mortem milk contained only about 90 per cent as high a percentage as the ante-mortem milk. Controlling the temperature of the udder did not prevent a reduction between the first and second post-mortem of about 10 per cent in percentage of solids not fat.

ASH

The percentage of ash showed a definite and consistent upward trend through the post-mortem milkings. (Table 4.) In every instance in both groups, the percentage of ash was higher in the first post-mortem than in the ante-mortem milk, and was still higher in the second than in the first post-mortem milk.

TABLE 4.—Percentages of ash in milk obtained from the same udders before death of the cow and after death and amputation of the udder

Group and cow No.	Ante-mortem milkings (average for 2 days)	First post-mortem milking	Second post-mortem milking	First post-mortem value as percentage of ante-mortem value	Second post-mortem value as percentage of ante-mortem value
Group 1:					
459	0.83	0.88	0.94	106.02	113.25
292	.75	.78	.82	104.00	109.33
123	.74	.80	.89	108.11	120.27
272	.76	.79	.85	103.95	111.84
Average	.77	.81	.88	105.52	113.67
Group 2:					
846	.78	.79	.83	101.28	106.41
903	.77	.78	.85	101.50	110.39
811	.74	.79	.86	103.76	116.22
257	.74	.78	.82	105.41	110.81
253	.77	.82	.86	106.49	111.69
443	.78	.81	.87	103.85	111.54
255	.74	.80	.87	108.11	117.57
Average	.76	.80	.85	104.74	112.09
Average for Groups 1 and 2	.76	.80	.86	105.03	112.67

TOTAL PROTEIN

For every individual in Group 1 the percentage of total protein in the first post-mortem milk was as great as that in the ante-mortem milk, or slightly greater. (Table 5.) In every instance, however, it was smaller in the second post-mortem milk than in the first.

TABLE 5.—Percentages of total protein in milk obtained from the same udders before death of the cow and after death and amputation of the udder

Group and cow No.	Ante-mortem milkings (average for 2 days)	First post-mortem milking	Second post-mortem milking	First post-mortem value as percentage of ante-mortem value	Second post-mortem value as percentage of ante-mortem value
Group 1:					
459	3.61	3.79	3.63	104.99	100.55
292	2.35	2.35	2.09	100.00	88.94
123	3.21	3.22	3.19	100.31	99.38
272	3.31	3.39	3.22	102.42	97.28
Average	3.12	3.19	3.03	101.93	96.54
Group 2:					
846	4.03	4.00	3.59	99.26	89.08
903	4.69	4.51	4.10	96.16	87.42
811					
257	2.63	2.60	2.47	98.86	93.92
253	3.37	3.60		106.82	
443	3.32	3.39	3.19	102.11	96.08
255	2.60	2.72	2.66	104.62	102.31
Average	{ 3.45 3.44	{ 3.44 3.47	{ 3.20 3.20	{ 100.20 101.31	{ 93.76 93.76
Average for Groups 1 and 2	{ 3.31 3.31	{ 3.33 3.36	{ 3.13 3.13	{ 100.97 101.56	{ 95.00 95.00

* Data for cow 253 excluded.

† Data for cow 253 included.

In Group 2 the total protein content was not determined for any of the samples from cow 811 or for the second post-mortem milk from cow 253. The average percentages showing the relation of total protein in post-mortem and ante-mortem milk, when based on complete data for the five cows in Group 2, are almost the same as for those in Group 1. The percentage of total protein in the first post-mortem milk appeared to be approximately normal, whereas that of the second post-mortem milk was slightly below normal. This relationship was not materially affected by controlling the temperature of the amputated udders.

As previously stated, the butterfat test showed a downward trend from the ante-mortem to the first post-mortem milking. From the first post-mortem to the second post-mortem milking the trend was sharply downward for Group 1, upward for Group 2, and slightly downward when the averages for Groups 1 and 2 were combined. The percentage of total protein was relatively uniform in the three milkings. Under these circumstances the fat-protein ratio, which is determined by dividing the percentage of butterfat by that of the total protein, would of necessity follow a trend similar to that of butterfat. The fat-protein ratio, which usually is fairly stable in cows' milk, has been determined for the ante-mortem and for the first and second post-mortem milk from each cow, by dividing the percentage of butterfat by that of the total protein. The results are given in Table 6. The milk from every cow in the first group showed a decline in the fat-protein ratio from the ante-mortem to the first post-mortem milking, and an additional decline from the first post-mortem to the second post-mortem milking.

TABLE 6.—*Fat-protein ratios for milk obtained from the same udders before death of the cow and after death and amputation of the udder*

Group and cow No.	Ante-mortem milk	First post-mortem milk	Second post-mortem milk
Group 1:			
459.....	1.590	1.187	0.441
292.....	1.247	.545	.335
123.....	1.692	.994	.564
272.....	1.130	.531	.289
Average.....	1.415	.814	.407
Group 2:			
846.....	1.012	.675	.816
903.....	.870	.428	.537
811.....			
257.....	1.076	.477	.538
253.....	1.080	.583	
443.....	1.277	.590	.887
255.....	1.285	.636	.977
Average.....	{ a 1.104 b 1.100	.561 .565	.751 .751
Average for Groups 1 and 2.....	{ a 1.242 b 1.226	.674 .665	.598 .598

a Data for cow 253 excluded.

b Data for cow 253 included.

In Group 2 the data are somewhat different. The milk from every cow in this group for which complete data are available showed a decline of almost one-half in the fat-protein ratio from the ante-mortem to the first post-mortem, but an increase from the first to the second post-mortem milking. In the case of cow 811 the ratios could not be

determined, and in the case of cow 253 the ratio for the second post-mortem milk was not available.

LACTOSE

The percentage of lactose in the milk was determined by subtracting the sum of the percentages of fat, ash, and total protein from the percentage of total solids.⁶ Variations in lactose percentages were less consistent than for the other constituents. In Group 1 there was a strong tendency for the lactose content to be high in the first and low in the second post-mortem milk. (Table 7.) The average percentage of lactose in the first post-mortem milk was 103.91, whereas that in the second post-mortem milk was only 87.81 per cent as high as that in the ante-mortem milk.

TABLE 7.—Percentages of lactose in milk obtained from the same udders before death of the cow and after death and amputation of the udder

Group and cow No.	Ante-mortem milkings (average for 2 days)	First post-mortem milking	Second post-mortem milking	First post-mortem value as percentage of ante-mortem value	Second post-mortem value as percentage of ante-mortem value
Group 1:					
459	4.50	4.71	4.06	104.67	90.22
292	4.80	5.20	4.27	108.33	88.96
123	4.85	5.15	4.32	106.19	89.07
272	5.05	4.87	4.19	96.44	82.97
Average	4.80	4.98	4.21	103.91	87.81
Group 2:					
846	4.37	4.20	3.65	96.11	83.52
903	3.71	3.52	2.97	94.88	80.05
811					
257	4.49	4.09	3.17	91.09	70.80
253	4.36	4.00		91.74	
443	4.83	5.05	4.34	104.55	89.86
255	4.15	4.21	3.24	101.45	78.07
Average	{ * 4.31 * 4.32	{ 4.21 4.18	{ 3.47 3.47	{ 97.62 96.04	{ 80.42 80.42
Average for Groups 1 and 2	{ * 4.53 * 4.51	{ 4.56 4.50	{ 3.80 3.80	{ 100.41 99.55	{ 83.70 83.70

* Data for cow 253 excluded.

* Data for cow 253 included.

In Group 2, Table 7, the omissions for percentage of lactose are the same as for those of total protein in Table 5, and the averages are treated in the same manner. There was no upward trend in percentage of lactose from the ante-mortem to the first post-mortem milk, but the downward trend from the first to the second post-mortem milk was similar to that for Group 1. The percentage of lactose in the ante-mortem milk in Group 2 in general was lower than for Group 1. The lactose content of both the first and second post-mortem milk was not only lower for Group 2 than for Group 1, but was lower than that of the ante-mortem milk in Group 2.

In the combined averages for Groups 1 and 2, the first post-mortem milk appeared to be similar to the ante-mortem milk in percentage of lactose, but the second post-mortem milk was distinctly low in

⁶ In subsequent analyses of ante-mortem milk and of the first and second post-mortem milks obtained from three other amputated udders, both the deduction method and the polariscope were used in determining the lactose content. A downward trend from ante-mortem to first and second post-mortem milkings was shown, and it was somewhat more pronounced than the average for the 11 cows herein reported. When the deduction method was employed the percentages of lactose in the first and second post-mortem milkings were, respectively, 99.0 and 75.0 per cent as high as in the ante-mortem, whereas the percentages of lactose as determined with the polariscope were, respectively, 93.1 and 67.1 per cent as high as in the ante-mortem milk.

lactose. By groups, the quantitative reductions in percentages of lactose in the milk from the first to the second post-mortem milkings did not differ materially, but the reduction from the ante-mortem to the second post-mortem was considerably greater in Group 2 than in Group 1. In the former case they are 0.77 and 0.74 per cent, respectively, for Groups 1 and 2. In the latter case they are 0.59 and 0.84 per cent, respectively, for Groups 1 and 2.

SUMMARY OF ANALYSES

A summary of the data in Tables, 1, 2, 3, 4, 5, and 7 is presented graphically in Figure 1. Six separate graphs show the relation of the percentages of total solids, butterfat, solids not fat, ash, total protein, and lactose in the milk obtained at the first and second post-mortem milkings, to those in the milk obtained at corresponding ante-mortem milkings. The 100 per cent line represents the average composition of the ante-mortem milk, with which all other analyses are compared.

QUANTITY OF BUTTERFAT RECOVERED FROM AMPUTATED UDDERS AT AND SUBSEQUENT TO THE FIRST TWO POST-MORTEM MILKINGS

The greatest difference between the ante-mortem and the post-mortem milk was in the percentage of butterfat. On an average, the butterfat test of the post-mortem milk was only about half as high as that of the ante-mortem milk. This low test, together with the fact that only about 70 per cent of the milk was recovered, could only result in the recovery of a relatively small quantity of butterfat.

Table 8 gives for each cow, (1) the average quantity of butterfat in the ante-mortem milkings on the two days immediately before slaughter; (2) the total quantity of butterfat in both post-mortem milkings; and (3) the relation which the latter bears to the former expressed in terms of percentage.

TABLE 8.—Quantity of butterfat recovered from amputated udders in two post-mortem milkings, expressed as percentage of ante-mortem yield

Group and cow No.	Average quantity of butterfat in ante-mortem milk	Quantity of butterfat in post-mortem milkings			Proportion of ante-mortem yield recovered in post-mortem milkings
		First	Second	Total	
Group 1:	<i>Pound</i>	<i>Pound</i>	<i>Pound</i>	<i>Pound</i>	<i>Per cent</i>
459.....	0.6629	0.4140	0.0171	0.4311	65.03
292.....	.0819	.0986	.0203	.1189	17.44
123.....	.5561	.1888	.0234	.2122	38.16
272.....	.8284	.1170	.0214	.1384	16.71
Average.....	{ .6823	.2046	.0206	.2252	* 34.34 ‡ 33.00
Group 2:					
846.....	.7477	.4158	.0987	.5125	68.54
903.....	.6337	.1737	.0572	.2309	36.32
811.....	.5444	.1602	.0138	.1740	31.96
257.....	.7451	.1500	.0432	.1932	25.93
253.....	.7918	.2636	.0493	.3129	39.52
443.....	.7992	.2370	.0948	.3318	41.52
255.....	.7764	.2794	.0429	.3223	41.51
Average.....	{ .7200	.2400	.0568	.2968	* 40.76 ‡ 41.22
Average for Groups 1 and 2.....	{ .7063	.2271	.0436	.2707	* 38.42 ‡ 38.33

* Average of percentages.

‡ Pounds recovered post-mortem divided by pounds recovered ante-mortem.

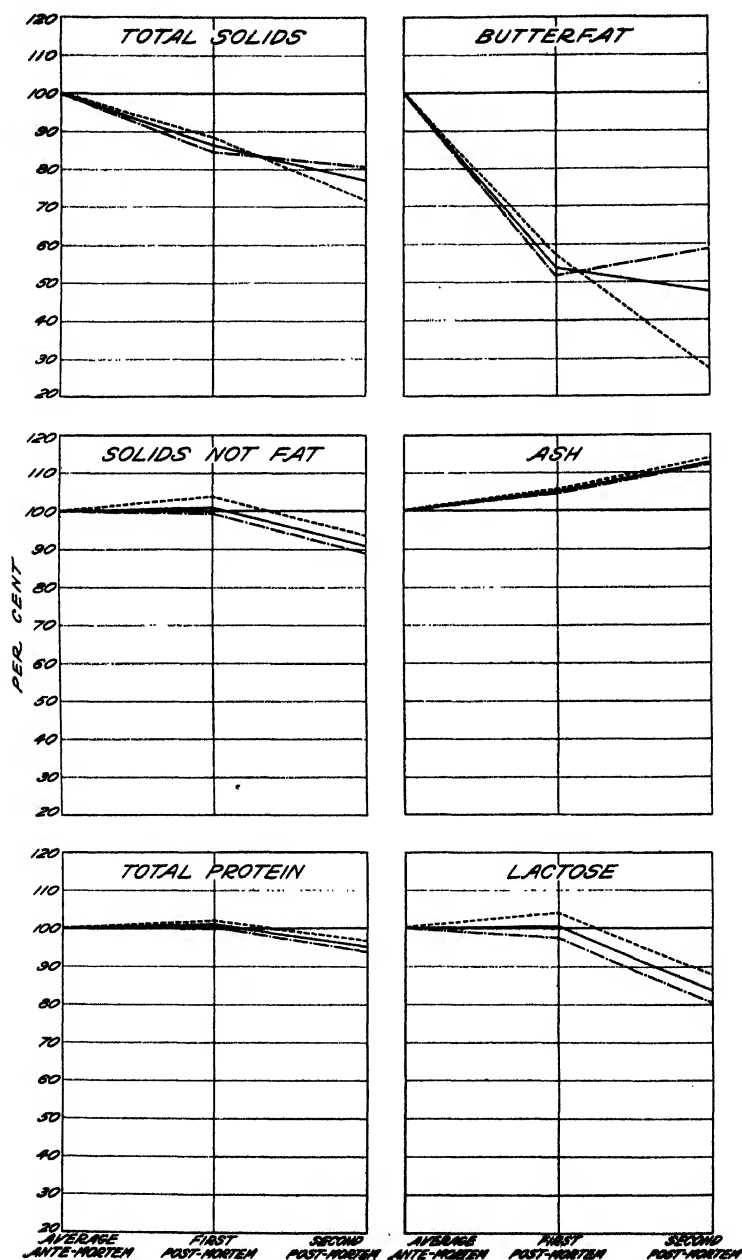


FIGURE 1.—Relative composition of the milk obtained from the udder before death of the cow, and in the first and second milkings of the amputated udder; expressed as percentage of ante-mortem composition. Dotted lines represent averages for cows of Group 1; broken lines represent averages for Group 2; and solid lines, for both groups combined

The low butterfat test and the consequent small quantity of butterfat recovered in the milk drawn from amputated udders, regardless of whether the udders were kept at blood temperature or allowed to become chilled, caused considerable speculation. Believing that a residue of butterfat might have remained in the amputated udder after post-mortem milking, the writers attempted to recover additional quantities through the use of solvents. It was believed that failure to recover fat in a solvent that had been injected through the teat into the secretory system of an udder and subsequently drawn out, should indicate that the tissues had not been affected and that the recovery of fat in solvents removed from lactating udders could be considered as butterfat.

The first preliminary trial was conducted with a nonlactating udder. Petroleum ether was introduced through the teats into the secretory system of two quarters of the udder. Removal of the solvent from one quarter was commenced five minutes after injection had ceased. From the other quarter the removal was commenced immediately. More than 2 gallons of the solvent was introduced into the two quarters, but after persistent manipulation of the udder only 1 pint, approximately, was recovered from each quarter. It appeared that, because of the volatility of this solvent, some of it permeated the tissues and escaped, but that the greater part was held within the finer divisions of the secretory system. Analysis showed that the 490 c c and the 488 c c of solvent recovered from the two quarters contained only 0.13 g and 0.19 g of fat, respectively.

Acetone was similarly introduced into the other two quarters of the same udder. Removal was commenced $9\frac{1}{2}$ minutes after one quarter had been filled and two minutes after the other had been filled. In the former case, the teat became hard and nothing could be milked out until it was opened at the end. The fluid then came freely in a stream. Apparently the acetone attacked the tissue rather severely, as some of the lining of the teat canal was discharged in shreds. In the other quarter the action of the acetone appeared to be less severe, and the recovery of the solvent was more readily accomplished. The quantities of acetone recovered from the two quarters were 760 c c and 517 c c. They contained only 0.51 g and 0.74 g, respectively, of fat.

In two other trials hot water was pumped into amputated udders. Water at 70° C. was introduced into a rear quarter of a slightly lactating udder, and withdrawal of the water was commenced immediately after the udder was filled, which required 10 minutes. About 5,400 c c was introduced, but only 955 c c was recovered. Water at 70° apparently affected the tissues and caused a portion of the lining of the teat canal to slough off and come out as a plug. The front quarter of the same udder was filled with water at 40°. Five minutes was required to fill this quarter, and removal of the water was commenced at once. The quantity introduced was 3,700 c c, but only 425 c c was recovered. Water at 40° appeared not to injure the tissues. The fat contained in these two recoveries of 955 c c and 425 c c was 1.33 g and 0.49 g, respectively. It appears that water at 40° is about as effective as that at 70° in recovering butterfat, and is relatively less injurious to the tissues of the udder.

In another trial, water at from 40° to 42° C. was introduced into a nonlactating udder. Filling of the rear quarter required 4 minutes and removal was commenced immediately thereafter. Filling the front

quarter required $3\frac{1}{2}$ minutes and removal of the water was commenced $1\frac{1}{2}$ minutes later. The quantity introduced was not measured. The quantity recovered from the two-quarters was mixed together and amounted to approximately 100 c c. It contained only 0.001 g of fat.

In an additional trial with a cow secreting a small quantity of milk, the udder was emptied as thoroughly as possible by hand milking and by inserting into the teat a milking tube attached to a vacuum pump. About 135 c c of milk was removed from the two left quarters in this manner. Approximately one-half gallon of petroleum ether was then pumped into the left front quarter. The vacuum pump was again attached to a milking tube in this quarter and 470 c c of the solvent was removed. Analysis indicated that this recovered solvent contained a total of 4.376 g of fat. As the specific gravity of the petroleum ether was 0.632, the solvent recovered contained approximately 1.47 per cent of fat by weight. As an indication of the relative efficiency of hand manipulation of the udder and of similar manipulation aided by a vacuum pump, this test is of interest. In the first trial with petroleum ether and removal by hand, about 13 per cent of the solvent was recovered. When the vacuum pump was used approximately 25 per cent was recovered. The fact that the first trial was made with a nonlactating udder, and the second with a slightly lactating udder, may have influenced these results.

After the solvent had been removed (by vacuum) from the left front quarter, 3,370 c c of cold water was pumped into the left rear quarter. By the use of the vacuum pump together with hand manipulation, 733 c c of the water was drawn out. On again comparing the efficiency of the hand method with the vacuum method, it appeared that when water was pumped into the udder, withdrawal by hand in two trials recovered 17.7 and 11.5 per cent of the quantity introduced. The vacuum method of removal recovered 21.8 per cent. All three tests were conducted, with udders secreting small quantities of milk.

These preliminary trials indicate (1) that petroleum ether as a solvent does not dissolve the tissue fat to any significant extent, and that its action on the udder tissue is less severe than that of acetone; (2) that the use of the vacuum pump in combination with hand manipulation is more efficient than hand manipulation alone in recovering from the udder the fluids previously injected into it; and (3) that not more than about one-fourth of the fluid injected was recovered by any method. The small recovery of fluid injected into the secretory system of the udder leads the writers to believe that a considerable quantity of milk may be left within the udder after the amputated udder has been milked out. This observation also strengthens the belief that nearly all the milk obtained at any milking is present in the udder at the time the milking process is commenced, even though the percentage recovered in some cases is less than in others and may be considerably less than 100 per cent of the quantity obtained at corresponding milkings before death.

As petroleum ether appeared to be a relatively efficient solvent for butterfat and comparatively nondestructive to udder tissues, it was selected for use in an attempt to recover additional butterfat from three amputated udders that had been subjected to two post-mortem milkings four hours apart. Before the solvent was introduced, the udder of cow 257 was subjected to a third post-mortem milking,

which yielded 0.4 pound of milk. Assuming that this milk contained 1.3 per cent butterfat, which is the average of the tests of the second-post-mortem milking from the two halves, the quantity of fat recovered was 0.0052 pound. Petroleum ether then was pumped through the teat into the right rear quarter for six minutes, and into the right front quarter for five minutes. Removal from each quarter was commenced within two minutes after the completion of the injection. Removal in this instance was entirely by hand manipulation. The quantity recovered from the two quarters was not measured, but it was found to contain 20.135 g of fat. Assuming that the other half of the udder would have yielded an equal amount of fat, the fat recovery in the solvent for the udder would have been 0.0888 pound.

The left half of the udder of cow 253 was similarly treated. A third post-mortem milking of the udder yielded 0.13 pound of milk containing 2.52 g, or 0.0056 pound of fat. Into each of the left quarters injection of petroleum ether was continued for three minutes. Removal was commenced at once. Hand manipulation was followed by application of the vacuum pump, and the total recovery of solvent appeared to be about half as much as the quantity introduced. The solvent from two quarters of the udder contained 2.225 g of fat, indicating a probable recovery in the solvent of 4.45 g or 0.0098 pound from the udder.

The experiment was repeated with the udder of cow 443. A third post-mortem milking yielded 0.36 pound of milk containing 10.75 g or 0.0237 pound of fat. Petroleum ether was introduced into the two left quarters. Injection was continued for two minutes in each quarter. Hand manipulation and employment of the vacuum pump resulted in the recovery of 1,700 c c, or more than 43 per cent of the quantity injected. The solvent which was recovered contained 20.33 g of fat, indicating a probable recovery of 40.66 g or 0.0897 pound of fat from the entire udder.

If it be assumed that an amount of butterfat equal to that recovered in corresponding ante-mortem milkings was present in the udder of the cow when killed, the third post-mortem milking and the use of a solvent resulted in the recovery of 0.94 and 16.09 per cent, respectively, of the amount of butterfat calculated to be retained in the udder of cow 257 after completion of the first and second post-mortem milkings. Corresponding recoveries in the third post-mortem milking and in solvents were 1.17 and 2.05 per cent, respectively, for cow 253, and 5.07 and 19.19 per cent, respectively, for cow 443. The retention of the butterfat in the udder, after all attempts to recover it had been made was, on this basis, 0.46, 0.46, and 0.35 pound, respectively, for the three cows, which was equivalent to 61.45, 58.54, and 44.29 per cent, respectively, of the total quantity of butterfat present when the cow was killed.

A fairly definite idea of the effectiveness of an additional post-mortem milking followed by the use of a solvent, in recovering residual fat from amputated udders after two post-mortem milkings, may be obtained from Table 9.

TABLE 9.—*Recovery of butterfat subsequent to the second post-mortem milking of amputated udder*

Cow No.	Butterfat in ante-mortem milk	Total butterfat in milk obtained at first and second post-mortem milkings	Proportion of ante-mortem yield recovered in first and second post-mortem milkings	Butterfat recovered in third post-mortem milking	Fat recovered in solvent	Total fat recovered from all sources	Proportion of ante-mortem fat yield recovered from all sources
	Pound	Pound	Per cent	Pound	Pound	Pound	Per cent
257	0.7451	0.1932	25.93	0.0052	0.0888	0.2872	38.55
253	.7918	.3129	39.52	.0056	.0098	.3283	41.46
143	.7992	.3318	41.52	.0237	.0897	.4452	55.71
Average	.7787	.2793	^a 35.66 ^b 35.87			.3536	^a 45.24 ^b 45.40

^a Average of per cent values.^b Obtained by dividing total pounds of fat recovered by total pounds of fat in ante-mortem milk.

On an average, 35.66 per cent of the quantity of fat contained in the ante-mortem milk from these three cows was recovered in the first two post-mortem milkings. By adding a third post-mortem milking and subsequently employing a solvent, the butterfat recovery was increased from 35.66 per cent to 45.24 per cent of the quantity contained in the ante-mortem milk. The average post-mortem recovery of milk for the same cows was 74.03 per cent. Corresponding percentages, obtained by dividing the total number of pounds of butterfat recovered in the first, second, and third post-mortem milkings and in the solvent, by the average number of pounds of butterfat in the ante-mortem milkings, are 35.87 and 45.40, respectively.

SUMMARY AND CONCLUSIONS

The butterfat test of the milk obtained from amputated udders was, on an average, about half as high as that of the milk obtained from the same udders before death.

Keeping the udder at approximately blood temperature until after the completion of post-mortem milking had a distinct tendency to keep the butterfat test of the milk from the second post-mortem milking as high as, or slightly higher, than that of the milk from the first post-mortem milking. In the experiments with the cows in Group 1, where post-mortem temperatures of the udder were not controlled, the butterfat test was only about half as high in the first post-mortem milking as in the ante-mortem milking, and only approximately half as high in the second as in the first post-mortem milking. In the experiments with the cows in Group 2, where post-mortem temperatures of the udder were controlled, the butterfat test of the first post-mortem milking was only about half as high as that of the ante-mortem milking, but the milk obtained at the second post-mortem milking was higher than that of the first post-mortem milking in butterfat test.

All measures introduced for the purpose of controlling the conditions under which the cow was killed, or under which the udder was held after death, failed to increase the total recovery of butterfat in the post-mortem milking to more than about 40 per cent of the total butterfat yield that was secured in the ante-mortem milking. Keep-

ing the udder under controlled conditions resulted in the recovery of somewhat more butterfat in the second post-mortem milking than was the case when the udder temperatures were not controlled, but this increased recovery was not sufficient to increase materially the total amount recovered. Thus the percentage of butterfat in the milk was maintained and even slightly increased from the first to the second post-mortem milking in Group 2, where the udders were kept at blood temperature. The cause of the consistent decrease of nearly one-half in the butterfat test of the first post-mortem as compared with the ante-mortem milk has not been determined. (See reference to the suggested possible effect of the nervous shock produced by killing the cow and severing the nerves in the process of amputating the udder (8).)

Flushing out the secretory system of amputated udders with a solvent increased to some extent the total quantity of butterfat recovered. In trials with three udders, the addition of a third post-mortem milking, and a subsequent injection and removal of petroleum ether, increased the proportion of the total butterfat obtained from amputated udders, from 35.87 to 45.40 per cent of the quantity of butterfat in the milk obtained from the same udders at corresponding milkings before death.

If it be assumed that the amount of butterfat present in the udder when the cow was killed was equal to that recovered in corresponding ante-mortem milkings, the retention of butterfat in the three udders after three post-mortem milkings and subsequent flushing with petroleum ether, would be 0.46, 0.46, and 0.35 pound, respectively. These quantities are equivalent to 61.45, 58.54, and 44.29 per cent, respectively, of the total quantity of butterfat present when the cow was killed.

Not more than 44 per cent of the fluid injected into amputated udders was subsequently removed in any one of several trials, even when hand manipulation was combined with the use of a vacuum pump.

Neither the quantity of milk recovered from amputated udders nor its relative test were as high as those shown by Petersen and his associates (5). This recalls a previous comment (8) regarding the possible effect of milking one-half of the udder immediately before death, on the recovery of milk from the other half after death.

The percentage of total solids was distinctly lower in the first post-mortem than in the ante-mortem milk, and still lower in the second than in the first post-mortem milk. When the amputated udders were kept warm the decline in percentage of total solids from the first to the second post-mortem milking was greatly reduced. Since the butterfat is included in the total solids, such a tendency is to be expected.

The percentage of solids not fat was nearly the same in the first post-mortem as in the ante-mortem milk, but was distinctly lower in the second than in the first post-mortem milk. Controlling the temperature of the udder did not prevent the reduction in the percentage of solids not fat between the first and second post-mortem milkings.

The percentage of ash was higher in the first post-mortem than in the ante-mortem milk, and decidedly higher in the second than in the first post-mortem milk. This trend is shown for every cow in each

group and does not appear to have been affected by the temperature under which the udder was held after it was amputated.

The percentage of total protein was almost the same in the first post-mortem as in the ante-mortem milk, but it was lower in the second post-mortem than in either the ante-mortem or the first post-mortem milk. This trend is not so consistent in the individual experiments as in the case of ash, but averages almost the same regardless of the temperature at which the udder was held after amputation.

The fat-protein ratio, as anticipated, followed a trend very similar to that of the butterfat test. In Group 1 the average ratio for the first post-mortem milk was only slightly more than half of the average ratio for the ante-mortem milk. The ratio for the second post-mortem milk was almost exactly half of that for the first post-mortem milk. In Group 2 also, the reduction in ratio from ante-mortem to first post-mortem milk was about one-half, but the ratio for the second post-mortem milk was decidedly higher than that of the first post-mortem milk. The average fat-protein ratio for the first post-mortem milk in Groups 1 and 2 combined was slightly more than half that of the ante-mortem milk, whereas the ratio for the second post-mortem milk was slightly less than half of that for the ante-mortem milk.

When the udders were allowed to become chilled, the percentage of lactose was slightly higher in the first post-mortem milk and much lower in the second post-mortem milk than in the ante-mortem milk. When the udders were kept warm after amputation, the trend was almost the same, except that the percentage of lactose in the first post-mortem milk was slightly below instead of above that of the ante-mortem milk. When Groups 1 and 2 are combined, the percentage of lactose in the first post-mortem milk was almost the same as that in the ante-mortem milk, but the second post-mortem milk had a much lower percentage than the ante-mortem milk.

The results based on 11 experiments confirm those based on the first two (7), and show that the most outstanding difference between the milk obtained from the udder of the living cow and that obtained from the amputated udder of the same cow after death was in the butterfat test.

LITERATURE CITED

- (1) GAINES, W. L., and SANMANN, F. P.
1927. THE QUANTITY OF MILK PRESENT IN THE UDDER OF THE COW AT MILKING TIME. *Amer. Jour. Physiol.* 80:691-701.
- (2) GOWEN, J. W., and TOBEY, E. R.
1927. UDDER SIZE IN RELATION TO MILK SECRETION. *Jour. Gen. Physiol.* 10:949-960, illus.
- (3) ——— and TOBEY, E. R.
1928. SIGNIFICANCE OF THE CHEMICAL COMPOSITION OF THE SECRETING AND DRY MAMMARY GLAND TO MILK SECRETION. *Jour. Gen. Physiol.* 12:123-128.
- (4) PETERSEN, W. E.
1928. SOME ASPECTS OF THE PHYSIOLOGY OF MILK SECRETION WITH SPECIAL REFERENCE TO MILK FAT. *Amer. Assoc. Med. Milk Comms., Inc., and Certified Milk Producers' Assoc. America, Inc., Proc. Ann. Conf.* 22:134-144.
- (5) ——— PALMER, L. S., and ECKLES, C. H.
1929. THE SYNTHESIS AND SECRETION OF MILK FAT. I. THE TIME OF MILK AND FAT SECRETION. *Amer. Jour. Physiol.* 90: 573-581.

- (6) RAGSDALE, A. C., ELTING, E. C., GIFFORD, W., and BRODY, S.
1927. STUDIES IN MILK SECRETION: (A) TIME RELATIONS IN MILK SECRETION; (B) MECHANISMS REGULATING VARIATIONS IN THE COMPOSITION OF MILK. Missouri Agr. Expt. Sta. Bul. 256:63-64.
- (7) SWETT, W. W.
1927. RELATION OF CONFORMATION AND ANATOMY OF THE DAIRY COW TO HER MILK AND BUTTERFAT PRODUCING CAPACITY, UDDER CAPACITY AND MILK SECRETION. Jour. Dairy Sci. 10:1-14.
- (8) ——— MILLER, F. W., and GRAVES, R. R.
1932. QUANTITY OF MILK OBTAINED FROM AMPUTATED COW UDDERS. Jour. Agr. Research 45:————, illus.

THE ORIGIN, DEVELOPMENT, AND INCREASE OF CHLOROPLASTS IN THE POTATO¹

By WINONA E. STONE

Assistant Plant Pathologist, Vermont Agricultural Experiment Station

INTRODUCTION

Much work has been done on mosaic and chlorosis, diseases whose primary visible effect is on the chloroplasts of the host plant. Yet a thorough search of the literature brings to light very few references to the normal chloroplast of the potato (*Solanum tuberosum* L.) or of any other member of the Solanaceae. Every phase of the life history of normal chloroplasts has been described again and again in other plants, but these data when brought together appear to form a chaotic mass of irreconcilable facts. The question arises which, if any, of these data apply to the normal chloroplasts of the potato or other members of this group. Obviously no progress can be made in the study of diseased chloroplasts until a firm basis is laid in a knowledge of the healthy normal ones.

Though small, the nightshade family includes many plants of economic importance, such as the potato, tomato, pepper, eggplant, tobacco, and petunia. All these plants are affected by degeneration diseases of the chloroplasts. Since little work has been done on any of these plants, the potato was considered a suitable and representative member of the group in which to study the formation and division of healthy chloroplasts.

HISTORICAL REVIEW

Since the discovery of plastids in 1791, investigators have striven to settle the more important phases of their history, i. e., their origin, structure, and method of division.

There are three outstanding theories as to the origin of chloroplasts.

(1) Chloroplasts arise by the condensation and contraction of the cytoplasm.

(2) Chloroplasts arise from preexisting bodies. Schimper (25, 26, 27)² was able to trace a complete succession of stages in the life history of chloroplasts. Mottier (18) was aware of the fact that chloroplasts develop from small bodies but believed that these bodies were merely certain stages in the continuous life history of individual chloroplasts.

(3) Chloroplasts arise from chondriosomes. When the study of plastid origin was extended to the higher plants a new school was originated by Guilliermond (4). There can be no doubt as to the accuracy of the observation of the supporters of the chondriosome and primordia theories of plastid origin. The explanation of the variance of the results probably lies in the different types of material used. Many investigators have used water plants. Others have

¹ Received for publication Dec. 7, 1931; issued October, 1932.

² Reference is made by number (italic) to Literature Cited, p. 433.

used mosses, liverworts, and ferns. Much of the work on the higher plants has been confined to the roots, but these organs, of course, are not a suitable place to observe chloroplast origin and so can not be used for comparison. In reproductive organs the chloroplasts are not functioning as in a leaf. Thus the dissimilarity of the plants and the plant organs studied may account for the widely divergent results not only of the present investigation but of those which led to the formation of the chondriosome and primordia theories.

Gris (3), who, so far as the writer is aware, is the only investigator who has worked on the origin of the chloroplasts in the potato leaf, described the differentiation of chloroplasts in the cells of leaves of *Solanum tuberosum* and various other plants, such as *Hydrangea hortensia*, *Lilium album*, *Acanthophippium*, *Aucuba*, *Sempervivum*, and *Vanilla*. In the very young hexagonal cells of the potato leaf he found the nucleus surrounded by a green, granular network. In older leaves the cells were entirely filled with granular matter. This granular substance broke up into sections, at first polyhedral but later assuming the spherical shape of normal, mature chloroplasts. Gris also found that development did not take place simultaneously throughout the entire leaf cross section, but that all stages in their differentiation from the cytoplasm might be observed within a limited area.

No record has been found of any attempts to trace the development of chloroplasts from chondriosomes or primordia outside the cell in cell sap. In fact Prát (19) and Börger (2) considered plant juice unsuited to the culture of isolated tissues and cells. Kny (10) and Heitz (6) were able to observe activity in isolated tissues, the latter using Knop's solution. Lubimenko (13, 14, 15) and Priestley and Irving (20) isolated plastids, the latter in cell sap. In this case there was some manufacture of sugar.

Belzung (1) investigated greening in potato tubers and was convinced that the starch grains formed the chlorophyll-impregnated areas of the cells. He was also of the opinion that these bodies are not true chloroplasts in that they do not manufacture starch.

At least three methods of plastid division have been noted. That most frequently observed is a process of constriction or pinching. Mikosch (17) described a type whereby the color concentrated at the poles and the ends grew away from each other. Schmitz (28) observed distinct fission. Kozłowski (11) believes the so-called division stages are merely accidental pairings of chloroplasts.

ORIGIN AND DEVELOPMENT OF CHLOROPLASTS

ATTEMPTS TO CULTIVATE PLASTIDS

In an effort to cultivate plastids plant juice expressed from actively growing portions of young plants by a metal press was filtered through several layers of cheesecloth, filter paper, and infusorial earth. The filtrate then passed easily through a Jenkins bacteriological microfilter. The filtrate contained no bodies which could be detected with the microscope. It was kept in the diffused light from a south window. The liquid remained clear and uncontaminated indefinitely. The nonappearance of bodies was probably due to one of two things. Either the culture medium was unfavorable for the development of the primordia, or the primordia are too large to pass through the

Jenkins filter. Under these conditions these results alone do not justify the assumption that chloroplasts do not arise from primordia.

GREENING OF TUBERS EXPOSED TO LIGHT

Detached tubers were greened by exposure to light in a window. The coloration extended into the tubers for some depth, but most attention was given to cells directly beneath the corky layer.

Colorless cells contained numerous large starch grains. In green cells starch was present, but the grains were often capped at one end by a mass of green protoplasm of varying size. The size of these caps was inversely proportional to the size of the accompanying grain, that is, the smaller the starch grain the larger the accompanying green area. The green patch was merely a rim, a considerable mass, or in some cells entirely inclosed the smaller grains in a green sheath. Besides these green areas accompanying the starch other detached green masses of rather indefinite outline were observed. (Pl. 1, A and B.) The size of the starch grain caps and the independence of some of the green masses make it improbable that this process is a conversion of leucoplasts into chloroplasts. Leucoplasts are soon stretched by grains of storage starch to thin films. The independent green masses can hardly be thought of as transformed leucoplasts, nor do they have the structural characteristics of chloroplasts. They seem to be regions in the cytoplasm which have become green, i. e., in which the chlorophyll has developed under stimulus of light. This phenomenon was described as early as 1877 by Wiesner (29) as "chlorophyll tinging the cytoplasm," and Harper (5) has called attention to the fact that a delimiting membrane is not essential in a chloroplast.

Some cells entirely devoid of starch contained rather definitely formed green bodies, vacuolate, and of varying size and shape, although in general resembling each other. (Pl. 1, C.)

Cells from the ungreened inner layers contained intact starch grains. Cells from the next region toward the surface contained corroded starch grains, broken in fragments, yet retaining the original shape. In the next layer starch fragments were smaller and fewer, and for the most part contained in a colorless vesicle. Directly beneath the cork the cells contained very small similar structures, but the colorless vesicles were here replaced by green regions inclosing particles of starch. (Pl. 1, E.)

Greening in the cytoplasm of the cells beneath the cut exposed surface of a tuber is due to large starch grains being partially enveloped by green protoplasmic masses. The starch grain was often found lying in a large vesicle inclosing also the green mass of protoplasm. (Pl. 1, F.) In some instances the green area assumed the shape of definitely formed bodies with one large or many small vacuoles, sometimes so large and numerous as to burst the outer green covering. (Pl. 1, D.)

Guard cells on small tuberlike sprouts exposed to light contained green bodies, evidently filled with starch; similar bodies from tuberlike sprouts grown in the dark were colorless although containing starch. (Pl. 1, G and H.)

Pith cells from fleshy sprouts grown in the light contained bodies which resembled normal chloroplasts in form, size, and color; cells

from sprouts kept in the dark contained only colorless bodies with minute starch grains. (Pl. 1, I and J.)

Guard cells of leaf buds had a uniform green cytoplasmic coloration with no suggestion of concentration into restricted regions and no apparent relation to the starch grains. (Pl. 1, K.)

The wide variety in shape and structure of the green bodies in tubers and sprouts exposed to light discourages the idea of discovering from them any facts concerning the origin of normal leaf chloroplasts. Perhaps they are not really chloroplasts at all. (1) Their shape is variable, indefinite, and not sharply outlined. Chloroplasts are oval or spherical and sharply defined. (2) Their size is not constant; even in the same cell bodies vary within a wide range. Normal chloroplasts usually have a constant definite size for a given species. (3) Their structure shows a lack of organization. They often differ little in density from the surrounding cytoplasm, their green color serving as their only distinction. Chloroplasts give the impression of definite organization with the ability to perform certain functions. (4) No divisions of these bodies were found in any of the material examined. In the life history of normal plastids divisions may be observed under a variety of conditions. (5) Although some green areas contained vacuoles and starch grains they seemed to be only temporary organs whose functions would soon cease or be taken over by more efficient bodies, i. e., by the true chloroplasts of the plant proper.

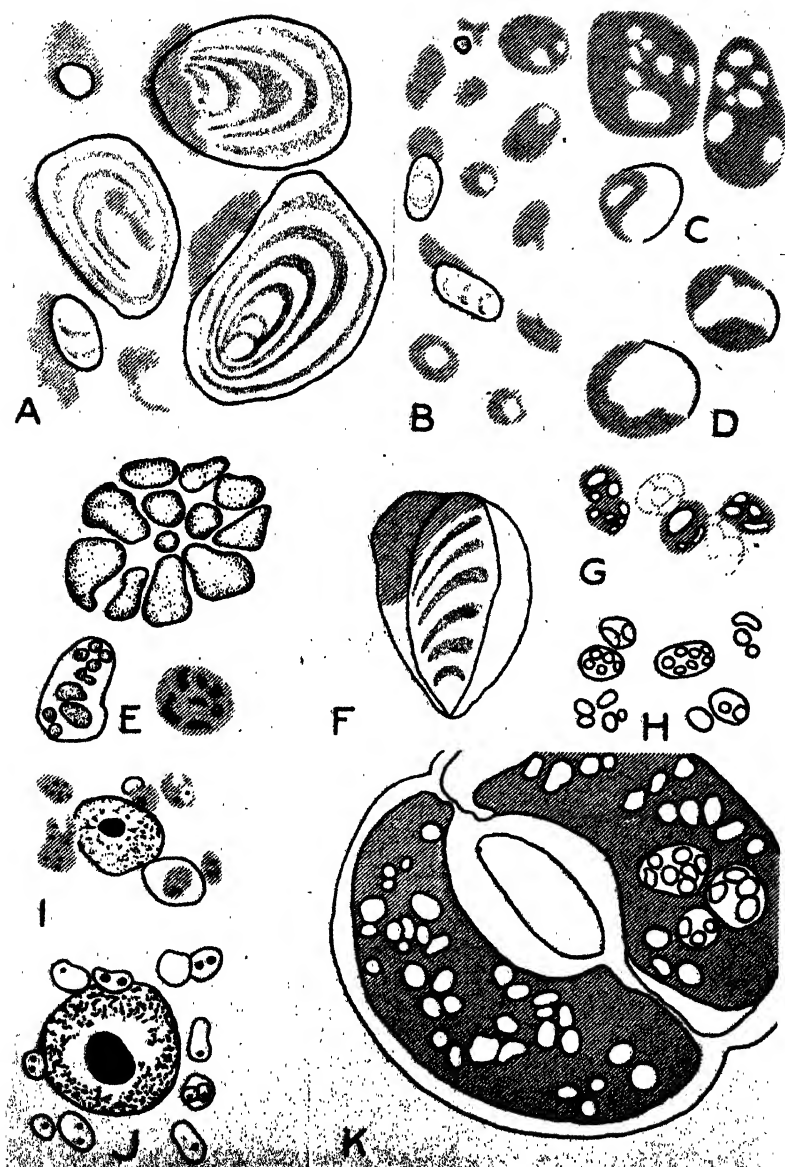
CHLOROPLASTS IN THE LEAVES

METHODS

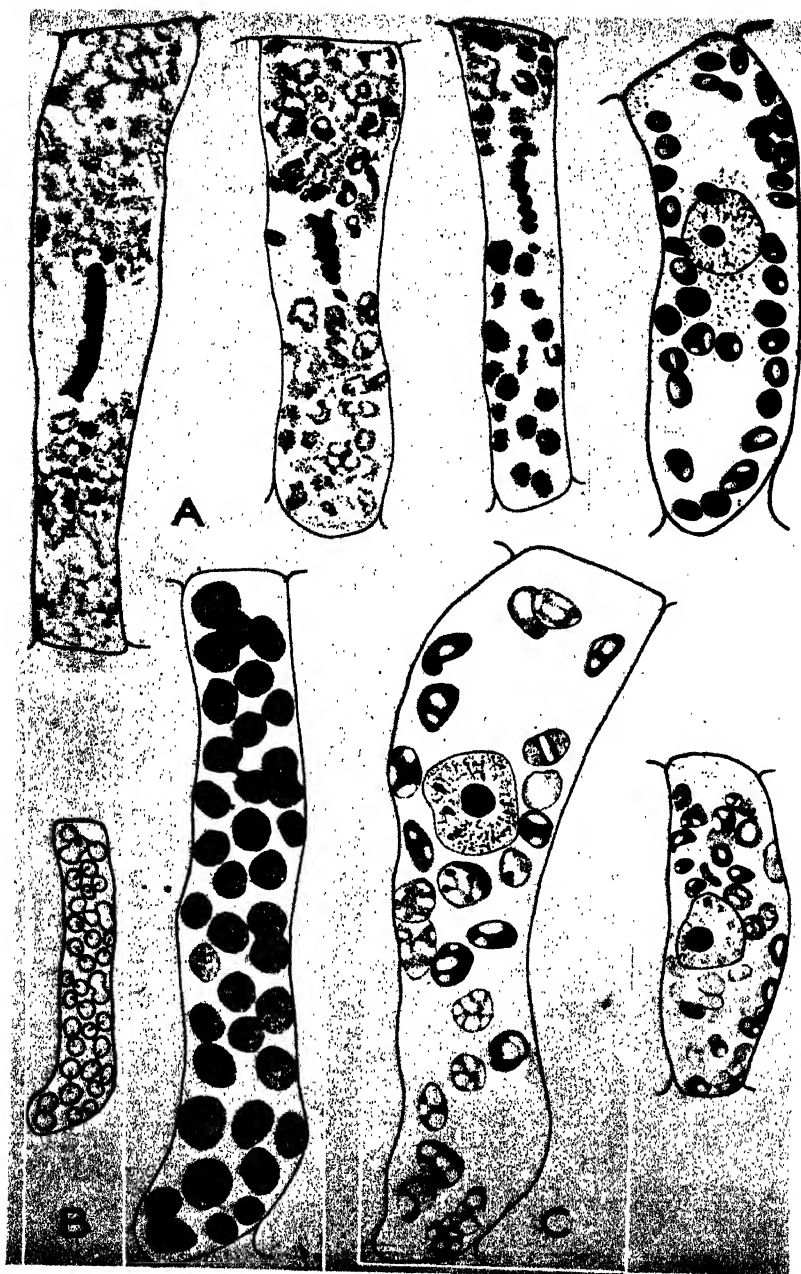
Material for the study of the origin and division of chloroplasts in potato leaves was obtained from plants grown in the greenhouse and out of doors. Fixations of leaves of different sizes from plants 1 to 4 inches high were made in Fleming's medium at all hours of the day and night in good growing weather. Sections 4 to 10 μ thick, according to the age of the leaf, were stained in either Flemming's triple or Heidenhain's iron-alum haematoxylin stains. In a search for development of chloroplasts from chondriosomes material was fixed in Benda's and Altmann's fixing solutions, as well as in Flemming's medium, and stained in Altmann's aniline-fuchsin or in iron-alum haematoxylin. Fixing fluids which contain acetic acid are claimed to be destructive to chondriosomes. Rudolph (22, 23), with a basic solution and a varying amount of acetic acid, found chondriosomes to be uninjured by concentrations of acetic acid equivalent to that in Flemming's solution. The results obtained with Altmann's or Benda's fixing fluids were quite unsatisfactory. The cells fixed in Altmann's fluid lacked any semblance of detail or differentiation. Benda's solution caused the chloroplasts to appear badly misshapen without the detail obtained by Flemming's fixing fluid followed by the iron-alum haematoxylin stain.

FORMATION OF PLASTIDS

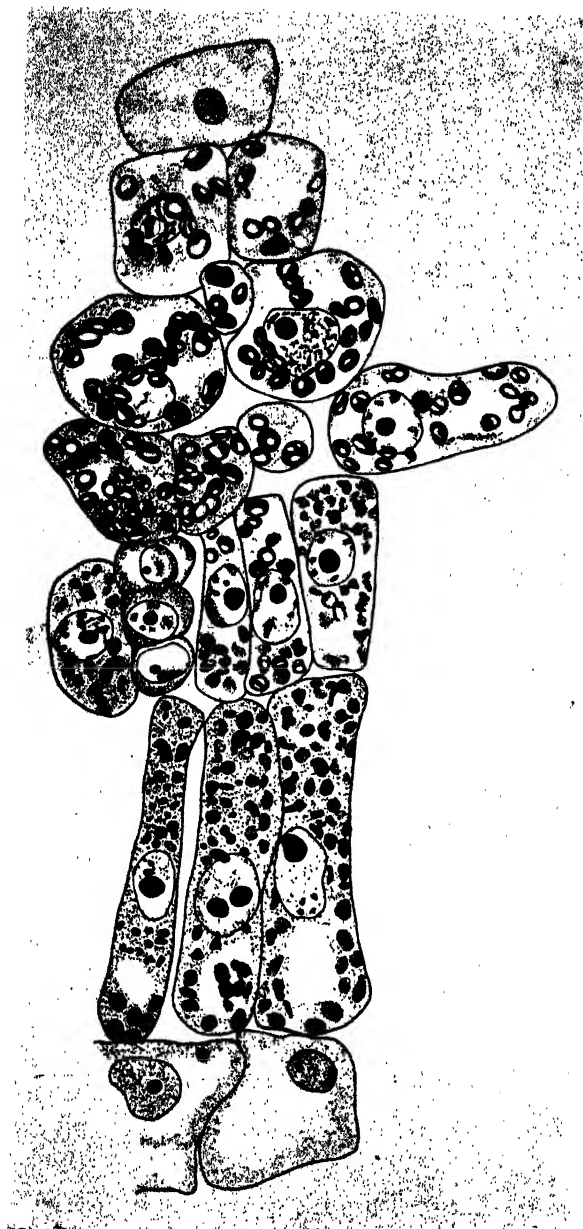
Formation of true plastids in the leaves differs greatly from that of the green bodies in exposed tubers. In stained sections of very young leaves, taken from the innermost part of growing tips, the cell cytoplasm appeared homogeneous and very dense. These leaves



Bodies from potato tubers and sprouts exposed to light or kept in dark. All $\times 1,450$. Shading represents green coloration. A to C, Green masses of cytoplasm from cells directly beneath skin of potato tuber exposed to light several days. D, Same as above, but from cells beneath new skin formed over cut surface. E, Bodies from three different depths of exposed tuber showing transition from disintegrating starch grain in deeper cell to greened body inclosing fragments of starch in cell directly beneath skin. F, Starch grain inclosed in vesicle and accompanied by green mass from new skin formed over cut surface of tuber exposed to light many days. G, Green bodies from hyathode of tuberlike sprout kept in light many days. H, Same from tuber kept in dark. I, Green bodies from pith cell of petiole of sprout exposed to light; starch stained with iodine. J, Same from sprout kept in dark. K, Stoma from leaf on young sprout, exposed to light; note general diffusion of chlorophyll throughout cytoplasm.



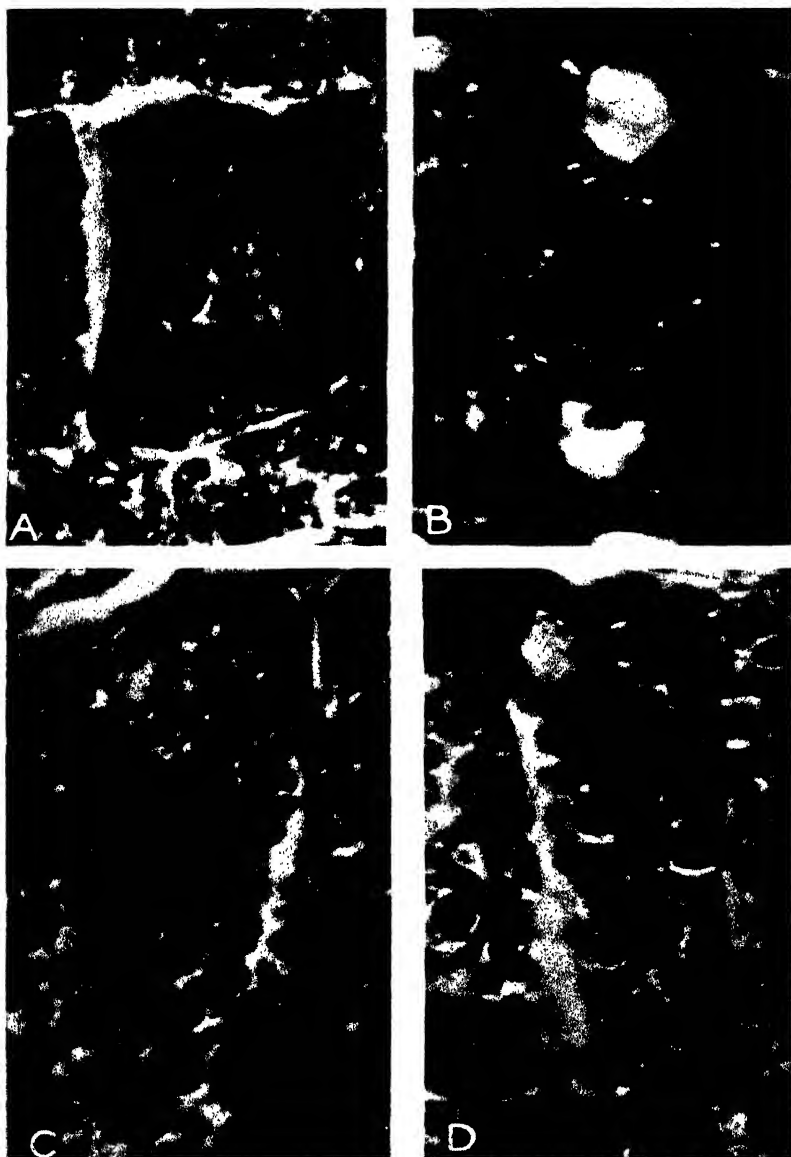
A, Palisade cells showing development of plastids from vacuolated cytoplasm to mature bodies. $\times 2,900$, 10 p. m. fixation. B, Palisade cell containing 11 plastids in the process of division and the rest in a very distinct pattern of pairs (right). $\times 2,900$. Plan of plastid divisions and pairing (left). $\times 1,300$, 6 a. m. fixation. C, Palisade cells from old leaf on left and young leaf on right showing variation in size of cells and plastids. No cytoplasm apparent in older cell and plastids more vacuolate than in the young cell. In young cell some plastids are just emerging from the ground cytoplasm. $\times 2,900$, 6 p. m. and 1 p. m. fixation



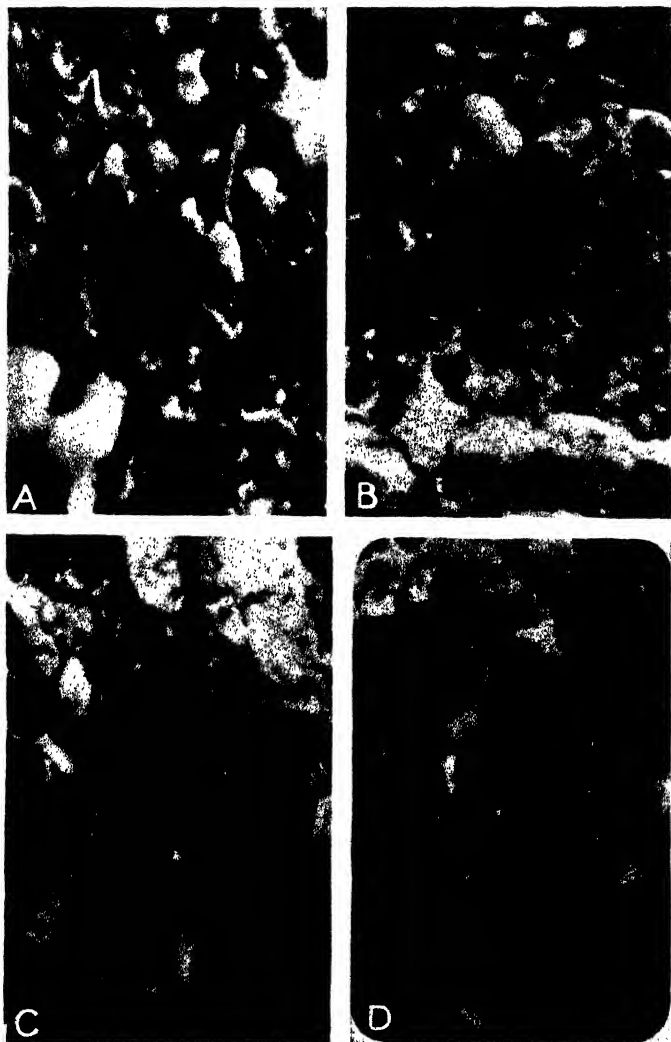
Section of a young leaf showing variation in degree of differentiation of plastids in spongy tissue and palisade cells, 11 p. m. fixation. $\times 2,000$



Photomicrograph of section through young leaf showing variation in degree of differentiation of plastids in spongy tissue and palisade cells, 10 p. m. fixation. $\times 1,100$



Stages in the process of plastid differentiation in palisade cells. $\times 2,400$. A, Embryonic cell, cytoplasm filled with many small vacuoles, 3 a. m. fixation. B, A large vacuole at either end, cytoplasm concentrating in dense irregular patches, 4.30 a. m. fixation. C, Plastids somewhat more rounded, 12.30 p. m. fixation. D, Plastids nearly complete, some containing starch grain 10 p. m. fixation



Stages in the differentiation of plastids in cells of spongy tissue. $\times 2,400$. A, Embryonic cell, slightly vacuolate, 4.30 a. m. fixation. B, Appearance of one large vacuole, three distinctly outlined plastids evident, the rest of the cytoplasm contracting in dense masses, 9 p. m. fixation. C, More mature cell; plastids definite, some containing starch, 9 p. m. fixation. D, Same as C, 12.30 p. m. fixation

were green, so it may be assumed that the green coloring matter was diffused throughout the cell contents, and examination of fresh material confirmed this. Sections of very small fresh leaves show no oval plastids, nor, in fact, any definite bodies, but only a fairly uniform, dense, green cytoplasmic mass. The cytoplasmic chlorophyll gathers into specific delimited regions.

In slightly older cells many small vacuoles accompany regional changes in cytoplasmic density and give evidence that the next step in plastid formation is the cutting up of the chlorophyll-impregnated cytoplasm. Vacuoles appear not only between the masses but within them as well. As the cells mature the cytoplasm concentrates, first in irregular patches, then in definite, regular, uniform shapes. During the rounding off masses are often held together for a time by cytoplasmic connections which disappear as the plastids become fully formed. Even in its very earliest form, as a shapeless mass of cytoplasm slightly denser than the surrounding cytoplasm, the chloroplast "anlage" is of considerable size. The process is one of concentration and contraction rather than of expansion and growth, so these original patches may be even larger than the resulting fully developed chloroplasts. (Pl. 2, A.)

Plate 3 shows development of chloroplasts in palisade cells. The young embryonic cell (A), short in proportion to its width, and with the nucleus taking up a large part of the contents, shows homogeneous cytoplasm, although a few tiny vacuoles may appear. These vacuoles become more pronounced in slightly older cells. B shows the two larger vacuoles in the ends of the cell, which are very characteristic of cells at this stage. The cytoplasmic knots are becoming more distinct and are taking on the appearance of immature plastids. These plastids (C and D) condense into the mature organs. There is no evidence of nor need for the intervention of any bodies, either chondriosomes or primordia; the whole process appears to be simply a cutting out of chloroplasts from the homogeneous cytoplasm. The plastids arise directly from cytoplasmic regions impregnated with chlorophyll.

In practically all the cells shown in Plate 3 the nucleus is in the process of dividing. There is no direct relation between plastid development and nuclear division; both processes take place in the early life of the cell. In the nearly mature palisade cells (D) a few plastids appear to be elongated as though about to divide, and many contain starch grains.

DIFFERENTIATION OF PLASTIDS IN DIFFERENT PARTS OF THE LEAF

Plastid differentiation in the cells of the spongy tissue of the potato is similar to that in the palisade parenchyma. (Pl. 4.) Large vacuoles appear and very dense regions of the cytoplasm can be easily distinguished (A). In B a large vacuole and in one section of the cell at least three distinct chloroplasts appear. In C and D the chloroplasts are well differentiated and contain starch grains. Some are rather elongated and either have not yet rounded off or are about to divide. The chloroplasts of Plate 8, B, are mature with sharp definite outlines and take a dense black stain. In Plate 4, C, and D, and Plate 8, B, many chloroplasts are surrounded by conspicuous clear zones. The nuclei of these spongy parenchyma cells are in

some phase of mitosis. Divisions occur in all planes. In most cases the distribution of the plastid material seems to be such that the new cells resulting from this division receive equivalent amounts.

Differentiation of the cytoplasm is not simultaneous throughout the leaf. In younger leaves the midrib is larger in proportion to the leaf lamina than in older leaves. Rib cells show rounded mature chloroplasts while the lamina cells contain only homogeneous protoplasm or early stages of breaking up. Spongy parenchyma cells show differentiated plastids before the palisade cells. Although spongy parenchyma plastids develop more rapidly than palisade plastids, differentiation is not complete throughout the spongy tissue before it starts in the palisade cells. Consequently a leaf section may show patches of densely stained mingled with patches of faintly

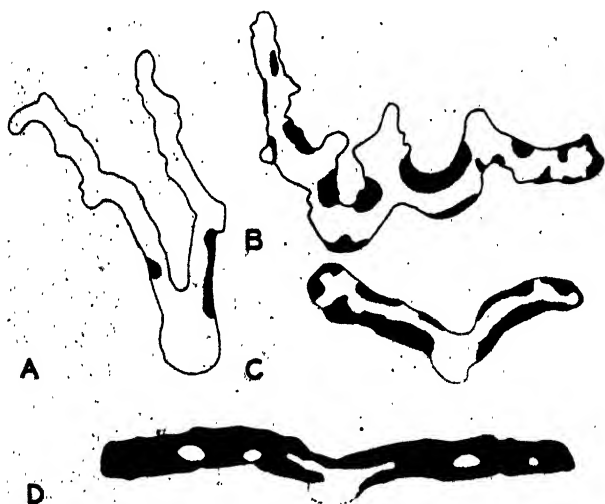


FIGURE 1.—Diagrammatic cross sections of leaves showing progressive differentiation (in order lettered) of plastids (in black). $\times 35$

stained plastids within both spongy and palisade tissues. The spongy tissue is filled with densely stained plastids while the palisade parenchyma still contains patches of faintly stained ones. (Fig. 1.) Even within individual cells differentiation of plastids occurs first in certain regions and not uniformly throughout the whole cell. Usually plastids nearer the epidermis or around the cell wall stain more densely than those of the lower half or center of the cell. (Pls. 5 and 6.)

In the midrib fully developed plastids often contained starch grains so large that the chloroplast itself was stretched to a mere shell surrounding the starch. (Fig. 2.) In both spongy and palisade tissues plastids were of two sorts, one densely staining, uniform throughout or containing a few small starch grains, the other indefinitely outlined and faintly staining. (Pl. 6.) In the two rows of palisade cells the nuclei are prominent but the chloroplasts are very indistinctly defined; in the upper row they appear as denser portions

of the cytoplasm with no definite shape; in the lower row they are somewhat more distinct. None retain the stain but appear as faint gray patches. The chloroplasts of the spongy parenchyma stain very densely, as intensely black, regular bodies, distinctly independent of the surrounding cytoplasm, in some instances apparently undergoing division. These cells are less compactly filled with plastid material than are the palisade cells, and the nuclei are less prominent. The palisade cells reach their normal mature state more slowly than do the cells of the spongy tissue. Zirkle's (31) similar variation in plastid-staining reaction in striped (variegated) corn was ascribed to the fact that some plastids were degenerated or permanently undeveloped, while others were normal, the uneven staining not being an indication of stages in progressive development. Ma (16) attributed this variation in the staining reaction in *Isoetes* to the condition of the starch within the plastid, that is, whether starch was being formed or hydrolyzed. The staining reaction of potato plastids seems rather to depend on the degree of development of the plastids themselves. If this reaction were dependent on the formation and hydrolysis of starch, it would recur with some regularity and frequency. No explanation can be given of this irregular progress of the development of the plastids in the potato-leaf cells.

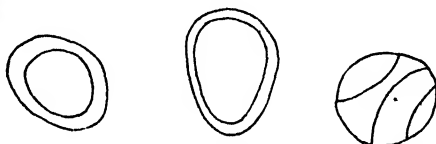


FIGURE 2.—Shell-like chloroplasts of midrib. $\times 4,200$

CHLOROPLASTS AND CHONDRIOSOMES

The description which has just been given of the development of the leaf chloroplasts leaves no place for the theory of their growth from small bodies in embryonic cells. Since the chondriosome has acquired such importance in the recent literature on chloroplast formation, special attention was given to the sections with chondriosomes in mind. No bodies resembling chondriosomes have been observed. Some investigators have found these bodies not only in young cells where chloroplasts were forming but also, in smaller numbers, in mature cells. Even in the youngest potato cells no chondriosomes were seen. The cytoplasm appeared homogeneous, granular, but free from rods, spheres, and threads. Young, recently differentiated, irregular plastids are somewhat smaller than those found in old leaves, but never so small that they would be mistaken for chondriosomes. The smallest chloroplasts seen were no less than 3μ in diameter; chondriosomes do not exceed 1μ in diameter.

RELATION OF NUCLEAR DIVISION TO PLASTID DIFFERENTIATION

The nucleus divides in the very young cell; at the same time the plastids differentiate in the cytoplasm; the two processes, while coincident, have evidently no relation to each other. Plastid differentiation extends over a longer period than is covered by active nuclear division, so that fully developed plastids are found only occasionally in a cell with a dividing nucleus. Plastids rarely divide until they are fully differentiated and definite in shape and staining qualities. Consequently plastid divisions are most abundant during the latest stages of nuclear division or after it has entirely ceased. (Pls. 3 and 4.)

The independence of plastids and nucleus may be further demonstrated by the indifference of the plastids to nuclear division with no migration of plastids or assembling about the nucleus. The plastids are distributed to the daughter cells by chance, as they happen to lie at one or the other end of the cell at the time of division, and equivalent numbers in the daughter cells are due to the distribution of plastids throughout the mother cell. (Pl. 8, C.) Zirkle (30) shows a concentration of the plastid primordia about the nucleus, but Ma (16), Kirby (9), and Randolph (21) failed to find any definite relation of plastid position to the dividing nucleus.

RELATION OF PLASTID NUMBER TO CELL GROWTH

At the time of plastid differentiation the cell is quite compactly filled with the chloroplasts; the cell grows rapidly in size, but the

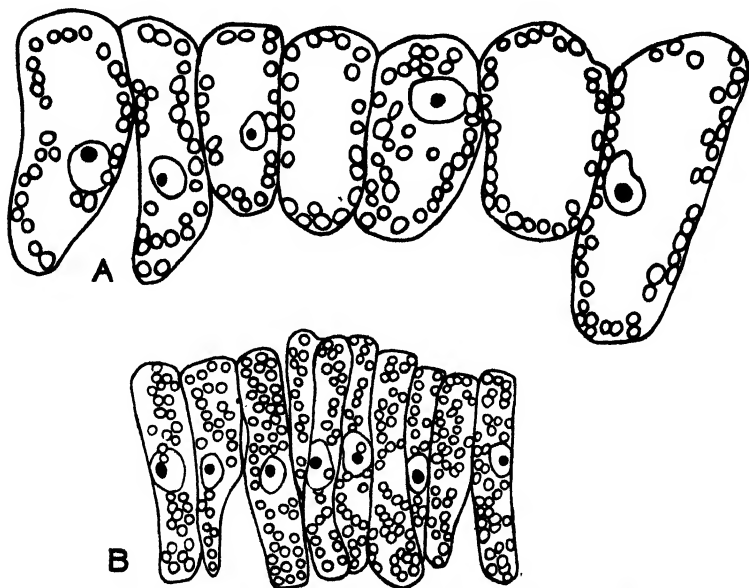
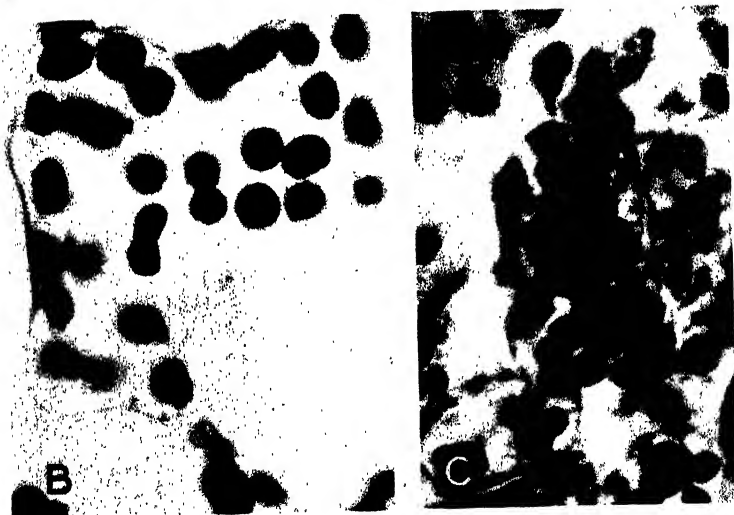
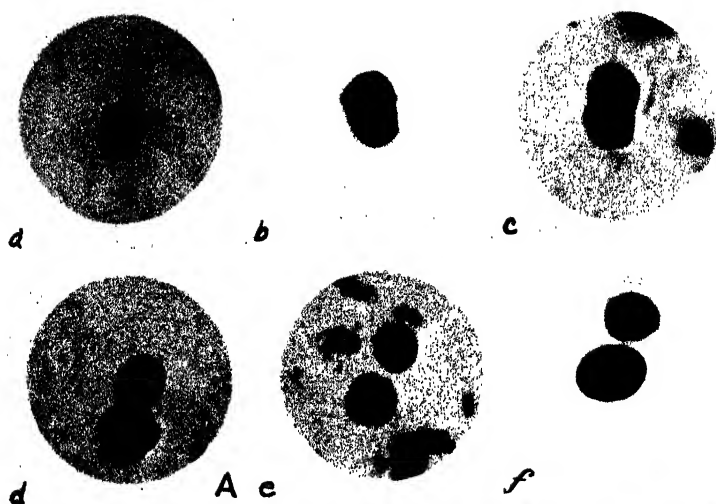


FIGURE 3.—Groups of palisade cells, old (A) and young (B). $\times 1,100$

plastids increase in size and number very slowly. An average of many measurements showed a cell size increase of 300 per cent to be accompanied by a 25 per cent increase in the size of the individual plastids. (Pl. 2, C.) But plastid divisions may cause a numerical increase of 45 per cent. The result of the inequality of the increase of volume of cells and plastids within the cell is shown by the small amount of the space within the older cell taken up by the plastids or other stainable material, as contrasted with that of well-filled young cells. In sections of cells from old leaves the chloroplasts are distributed for the most part only along the cell walls. (Fig. 3.) In a surface section the chloroplasts form single compact layers tightly appressed to the cell wall, the large cell lumen being occupied by the central vacuole. (Pl. 7.)



Surface section of mature palisade cells showing plastids lining the wall, leaving the central lumen free except for nuclei, 8 p. m. fixation, $\times 2,400$



A, Plastids which had become isolated from cells in sections. Successive steps in the elongation and division of an individual plastid to form two new plastids. Note starch in several plastids, and in *f* what is evidently the beginning of a second division. *a-e*, 11 p. m.; *f*, 4.30 a. m. fixation. $\times 2,400$. B, Young cell from spongy parenchyma. Plastids, mature, so distributed that each daughter cell will receive an approximately equal quantity, 11 p. m. fixation. $\times 2,400$. C, Region of spongy tissue showing variety of stages in division among neighboring plastids, even within the same cell, 10 p. m. fixation. $\times 2,400$

TABLE 1.—Measurements of drawings of 11 blocks of 8 cells each taken from leaves of various sizes and ages to compute number of plastids

Age of leaves	Area of block of 8 cells in square millimeters	Number of plastids	Plastids per 100 square millimeters
Very young -----	3,600	192	5
	4,125	270	7
	7,700	329	4
	7,728	368	5
	8,320	292	3.5
Older -----	8,980	320	3.5
	9,450	221	2
	10,200	392	4
	13,760	408	3
	14,400	496	3.5
Mature -----	31,140	544	1.7

In Table 1 are given measurements of drawings of 11 blocks of eight cells each taken from leaves of various sizes, ranging from those in which the plastids were barely differentiated to fully mature leaves. It can readily be seen that increase in plastid number does not keep pace with cell growth, for an increase of 765 per cent in cell area was accompanied by an increase of only about 185 per cent in plastid numbers.

TABLE 2.—Percentage increase in number of plastids as compared with percentage increase in cell size of young and of mature leaves

Kind of leaves	Increase in cell size	Increase in plastid number
Young -----	40	14
	90	20
	160	24
Mature -----	300	45

Table 2 (from another set of measurements) shows a consistent increase in plastid number corresponding, but not equal, to the increase in cell size. A large number of cells of various sizes were measured and the contained plastids were counted. Cells were then grouped together according to size. Using the group of smallest cells as a basis, the percentage of increase in cell size and plastid number was computed for each group.

The proportion of plastid volume to cell capacity does not actually reach such wide variance as the tables seem to indicate, since the plastids grow. In the youngest cells in which plastids were of definite, regular outline the average diameter of 30 plastids was 5.05μ . In the oldest leaves of this material the average diameter of 30 plastids was 6.43μ ; an increase of 27 per cent in the size of the plastids. Plastids of fully mature leaves from material which had been fixed from field-grown plants showed an average plastid diameter of 10.38μ or about 100 per cent greater than that of plastids in young leaves. (Fig. 4.) No comparison of cell areas in this material and the young leaf cells could be made, outlines of the old cell walls being too broken and vague.

PROCESS OF DIVISION

Plastid division in the potato leaf follows closely that described for other plants by early workers such as Kny (10), Sachs (24), and Nageli (18a). The gradual growth in the size of the plastid and the

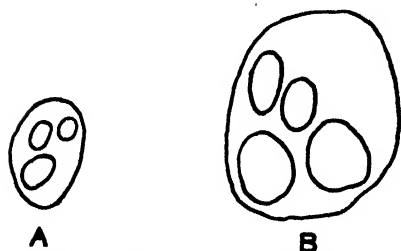


FIGURE 4.—Average plastid from young (A) and old (B) leaves. $\times 4,200$

change of shape from round to oval (fig. 5, A and B) continues, especially in the longer axis, until the plastid is about twice its original length. A constriction midway between the ends of the elongated plastid followed by a gradual contraction or pinching in of the plastid at this point results in the formation of two new plastids approximately the same size as the original. (Fig. 5, C, D, E, F.) Dividing

plastids may be homogeneous throughout or may contain one or more starch grains. (Pls. 8, A, and 9, C.)

In some cases dividing chloroplasts may show a clear zone in the region of the constriction (fig. 5, E), the contents becoming concentrated in the two halves. The material in each half rounds off, leaving a membrane between which connects the two new plastids for a time. This bridge may be nearly as wide as the diameter of the plastid or it may be a scarcely visible thread. (Fig. 5, D, E.) This thin

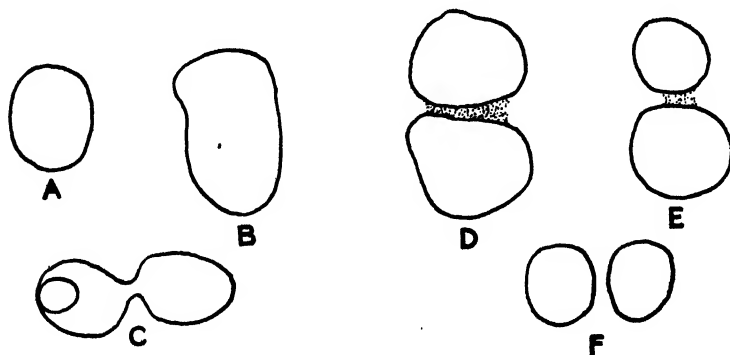
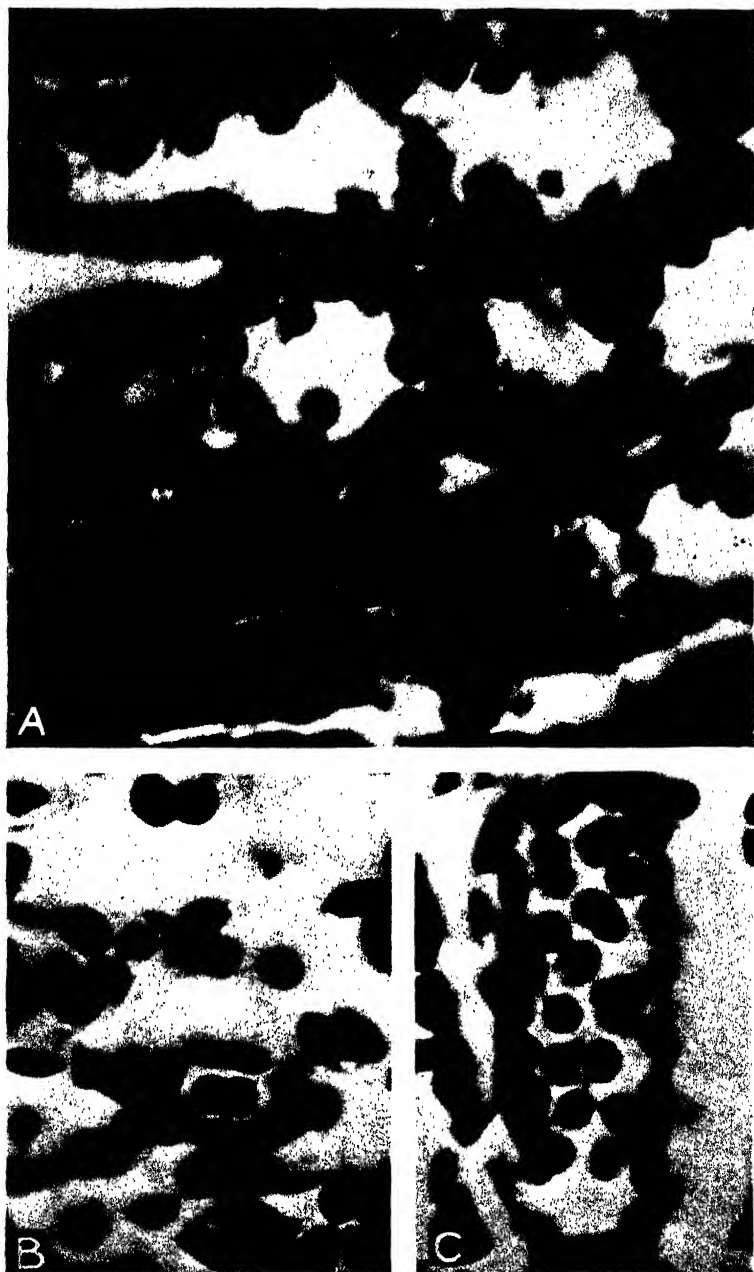


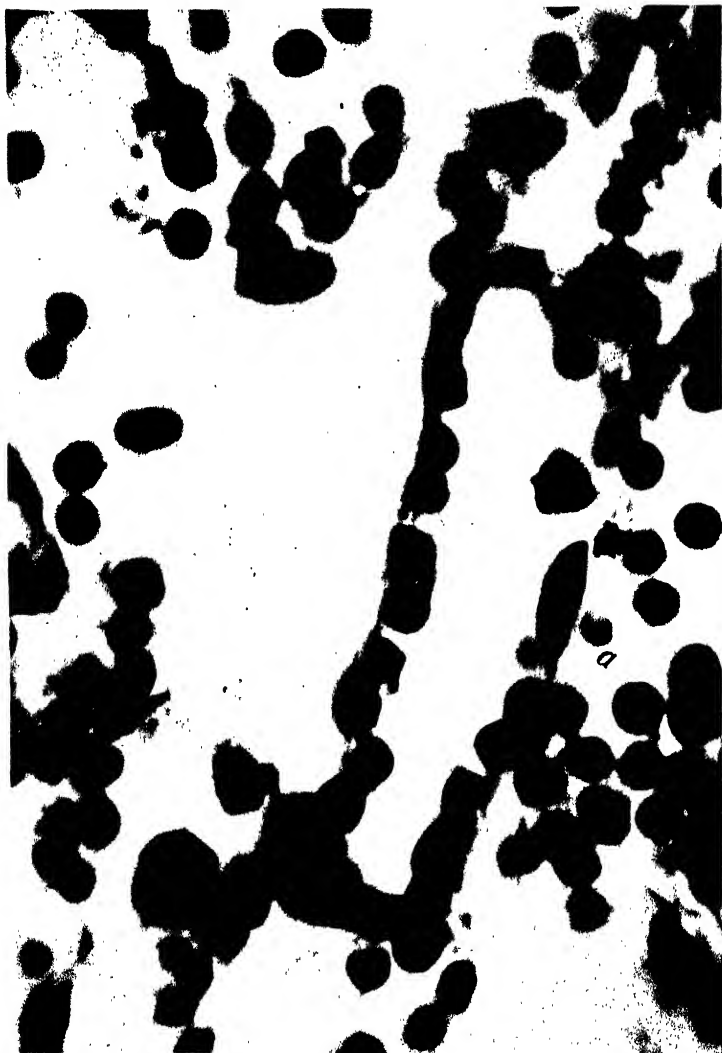
FIGURE 5.—A, Resting plastid; B, constriction of elongated plastid; C-E, various types of connections; F, joining not visible but clearly plastids resulting from division. $\times 4,200$

connection finally disappears and two independent chloroplasts result. Even though no longer joined, the new plastids remain in the same position with reference to each other, so that often in one field the plastids occur in distinct pairs. (Pls. 2, B, 9 and 10.)

Photomicrographs of stained material of division stages in plastids isolated from cells (pl. 8, A) show, with one exception, all chloroplasts with visible starch grains. Elongation and cutting in two of a chloroplast (pl. 8 A, b, c, d) are followed by two newly formed plastids no longer visibly connected but in the same position in which division occurred. (Pl. 8, A, e.) The position of members of some groups of



A, Palisade cells showing various plastid divisions within the same or neighboring cells, 6 a. m. fixation. $\times 2,400$. B, A section of spongy parenchyma showing many stages of plastid division, 3.30 p. m. fixation. $\times 2,400$. C, Palisade cells showing several stages of division in plastids; note starch in plastids, 11 p. m. fixation. $\times 2,400$



Palisade cell showing divisions in direction of long axis of cell and newly formed plastids arranged along cell wall. Many other stages in outlying regions. Note rosettelike pattern at *a*, 2 a. m. fixation, $\times 2,400$

plastids suggests the division of those resulting from a former division. (Pl. 8, A, f.) Heitz (6) found plastid division repeated so rapidly in severed moss leaves that the new plastids divided again in a plane at right angles to the first division before the first division was complete.

RELATION OF STARCH TO DIVISION

It was found that any starch grains present in a dividing plastid are apportioned to the new plastids as they happen to lie with respect to the line of division, thus confirming the findings of Sachs (24). (Fig. 6.) The amount of starch contained may vary from none to 1 to 3 large grains or 6 to 8 small ones. The starch is usually evenly distributed so that after division the newly formed plastids receive relatively equal amounts. Starch grains do not appear to hinder division. (Pls. 3, D; 8, A; and 9, C.)

DIURNAL PERIODICITY IN PLASTID DIVISION

Lange (12) found a daily periodicity in nuclear divisions, but a tabulation of the hours of day and night at which the material for the present study was fixed shows no such situation regarding plastid division. No attempt was made to count the plastid divisions in day and night material, but an examination of the photomicrographs shows that division is not confined to any particular hour but is going on at all times.

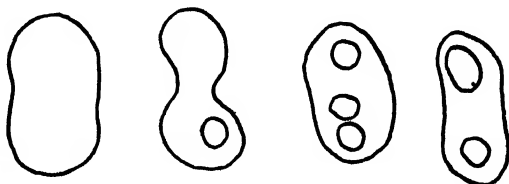


FIGURE 6.—Starch grains in plastids undergoing division. $\times 4,200$

Lower temperatures and relatively high humidity were associated with the hours of darkness but the initiation and rate of plastid division do not seem to be correlated directly with any external factors, such as light, temperature, or humidity.

DIVISION IN CHLOROPLASTS

PHASES OF DIVISION

Increase in plastid number results from the continuous division of the plastids originally differentiated in the cell. No period was determined for the maximum activity in division. Divisions have been found in the plastids which were scarcely fully differentiated (pl. 3, D); but were found in greater abundance in cells of leaves somewhat older. Often all the plastids in one cell were in some stage of division or were arranged in a distinct pattern of pairs as though they had just completed division. (Pl. 2, B.) In any one region all stages appear simultaneously (pls. 9 and 10); and even neighboring plastids may be in very different stages of division (pl. 10).

In young palisade cells with walls compactly lined with plastids divisions occur in a direction diagonal to the wall, so that one of the plastids of the newly formed pair is pushed into the central vacuole. (Pl. 2, B.) In old palisade cells, on the other hand, with walls sparsely lined with plastids, divisions occur parallel to the long axis of the cell

and newly formed pairs lie along the cell wall. (Pl. 10.) Several newly formed plastids may form a rosettelike pattern in which the inner faces of the plastids are angular. (Pl. 10, a.)

DISCUSSION

The phenomenon of plastid division has been observed in many plants. Sachs (24) suggested that plastid division followed elongation of the plastid and was occasioned by a stretching of the adjacent cell wall. Divisions occur, however, in plastids remote from walls and in planes which do not parallel that of the stretching of the cell wall. Heitz (6, 7, 8) claimed that plastid division was a result of the accumulation of products of assimilation. If this were the only factor involved divisions would be expected to occur most frequently when the plastids were filled with these products, i. e., during the day. Although plastid divisions were abundant in material fixed at all hours of the day and night, they were perhaps more plentiful in the material fixed during the night hours; in other words, when the plastids were losing the material temporarily stored during the hours of sunlight. The initiation of plastid division does not seem to be regulated either by the growth of the cell wall or by the accumulated food products. It appears rather to be one of the normal processes which taken together constitute cell growth.

Observations, measurements of cells, and plastid counts indicate that the number of plastids per cell is fairly constant. This being the case, plastid division is not independent of cell development but is merely one phase of the whole process. Cells of certain size contain a characteristic amount of chloroplast material divided into a fairly constant number of bodies which increases as the cell becomes larger.

SUMMARY AND CONCLUSIONS

An investigation of the origin of the chloroplasts in the potato (*Solanum tuberosum*) was made from the point of view of the three well-known theories of plastid origin in other plants.

Attempts to grow plastids from primordia outside the plant in expressed filtered leaf juice failed, either because plastids do not develop from primordia minute enough to pass through a Jenkins filter, because of the toxicity of the potato juice, or because the normal growing conditions in the cell were absent.

Tubers, greened by exposure to light, did not furnish satisfactory material for the study of the origin of the chloroplast. Greening is merely the result of the formation of chlorophyll in undifferentiated regions of the protoplasm. Although, after some time, these patches may assume a definite shape they can not be considered as typical chloroplasts.

A search for chondriosomes or plastid primordia in embryonic leaves by the use of recommended fixing fluids and stains revealed no bodies of this type. In embryonic leaves of the potato, plastids originate from the cytoplasm directly. By gradual vacuolation the cytoplasm is separated into dense masses which round off, become compact, and develop into mature, independent chloroplasts.

Differentiation of chloroplasts from homogeneous cytoplasm does not occur simultaneously throughout all portions of the leaf. Isolated

regions first appear in the spongy tissue, later in the palisade tissue. Differentiation is first completed in the spongy tissue. Plastids in the various regions of a single cell may be in quite different stages of differentiation.

Plastid differentiation seems to be only one phase of the series of changes which together bring about the maturing of a cell. Division of the nucleus occurs at the same time as plastid differentiation but independently. New cell formation usually reaches its maximum before plastid differentiation is complete. Plastids, even if formed, show no change of position during nuclear division.

Plastids increase in number as the cell grows, but the increase is much slower than the rate of cell growth. Increase in plastid size compensates somewhat for slow increase in plastid number.

Increase in plastid number is brought about by the division of the original chloroplasts.

Division of chloroplasts is a process of elongation and pinching. The connections between the new plastids may persist for some time. The arrangement of plastids in pairs is very striking even after all visible connections have disappeared. Plastid division figures, if present at all, are found in abundance throughout any one region of the leaf. In general all dividing plastids of a certain region are in approximately the same stage of division, although all stages may occur within a very limited area.

Plastid division seems to be a phenomenon connected with the growth and maturation of the cell.

LITERATURE CITED

- (1) BELZUNG, E.
1887. RECHERCHES MORPHOLOGIQUES ET PHYSIOLOGIQUES SUR L'AMIDON ET LES GRAINS DE CHLOROPHYLLE. *Ann. Sci. Nat., Bot.* (7) 5 : [179]-310, illus.
- (2) BÖRGER, H.
1925. ÜBER DIE KULTUR VON ISOLIERTEN ZELLEN UND GEWEBESFRAGMENTEN. *Arch. Expt. Zellforsch.* (1925) 2: 123-190, illus. [Abstract in *Bot. Centbl.* (n. F. 11) 153:195. 1927.]
- (3) GRIS, A.
1857. RECHERCHES MICROSCOPIQUES SUR LA CHLOROPHYLLE. *Ann. Sci. Nat., Bot.* (4) 7: [179]-219, illus.
- (4) GUILLIERMOND, A.
1911. SUR LA FORMATION DES CHLOROLEUCITES AU DÉPENS DES MITOCHONDRIES. *Compt. Rend. Acad. Sci.* [Paris] 153 : 290-292, illus.
- (5) HARPER, R. A.
1919. THE STRUCTURE OF PROTOPLASM. *Amer. Jour. Bot.* 6 : 273-300.
- (6) HEITZ, E.
1922. UNTERSUCHUNGEN ÜBER DIE TEILUNG DER CHLOROPLASTEN NEBST BEOBSACHTUNGEN ÜBER ZELLGRÖSSE UND CHROMATOPHOREN-GRÖSSE. 31 p. Strassburg.
- (7) ————
1925. DAS VERHALTEN VON KERN UND CHLOROPLASTEN BEI DER REGENERATION. *Ztschr. Zellforsch. u. Mikros. Anat.* (1925) 2: [69]-86, illus. [Reviewed by F. Weber in *Bot. Centbl.* (n. F. 6) 148: 129. 1925.]
- (8) ————
1925. EINIGE BEMERKUNGEN ÜBER CHLOROPLASTENTEILUNG UND CHLOROPLASTENGROSSE. *Biol. Zentbl.* 45 : 179-186.
- (9) KIRBY, K. S. N.
1928. THE DEVELOPMENT OF CHLOROPLASTS IN THE SPORES OF OSMUNDA. *Jour. Roy. Micros. Soc.* (3) 48 : 10-35, illus.
- (10) KNY, L.
1897. DIE ABHÄNGIGKEIT DER CHLOROPHYLLFUNCTION VON DEN CHROMATOPHOREN UND VOM CYTOPLASMA. *Ber. Deut. Bot. Gesell.* 15: 388-403.

- (11) Kozlowski, M.
1922. CRITIQUE DE L'HYPOTHÈSE DES CHONDRIOSOMES. *Rev. Gén. Bot.* 34: [641]-659, illus. [Reviewed by W. Riede in *Bot. Centbl.* (n. F. 3) 145: 385. 1924.]
- (12) LANGE, F.
1927. VERGLEICHENDE UNTERSUCHUNGEN ÜBER DIE BLATTENTWICKLUNG EINIGER SOLANUM-CHIMÄREN UND IHRER ELTERNARTEN. *Planta Arch. Wiss. Bot.* 3: [181]-281, illus.
- (13) LUBIMENKO, V.
1926. RECHERCHES SUR LES PIGMENTES DES PLASTES ET SUR LA PHOTOSYNTHESE. PREMIÈRE PARTIE: LA PHYSIOLOGIE DES PLASTES. *Rev. Gén. Bot.* 38: [307]-328, [381]-400, illus.
- (14) ————
1927. RECHERCHES SUR LES PIGMENTES DES PLASTES ET SUR LA PHOTOSYNTHESE. DEUXIÈME PARTIE: LES PIGMENTES DES PLASTES ET LEUR TRANSFORMATION DANS LES TISSUS VIVANTS DE LA PLANTE. *Rev. Gén. Bot.* 39: [547]-559, [619]-637, [698]-710, [758]-766, illus., 1927; 40: [23]-29, [88]-94, [146]-155, [226]-243, [303]-318, [372]-381, 1928.
- (15) ————
1928. RECHERCHES SUR LES PIGMENTES DES PLASTES ET SUR LA PHOTOSYNTHESE. TROISIÈME PARTIE: LA BIOLOGIE DE LA PHOTOSYNTHESE. *Rev. Gén. Bot.* 40: [415]-447, [486]-512, illus.
- (16) MA, R. M.
1928. THE CHLOROPLASTS OF ISOETES MELANOPODA. *Amer. Jour. Bot.* 15: 277-284, illus.
- (17) MIKOSCH, C.
1877. UEBER VERMEHRUNG DER CHLOROPHYLLKÖRNER DURCH THEILUNG. *Osterr. Bot. Ztschr.* 27: [41]-55, illus. [Abstract in *Just's Bot. Jahresber.* 5: 310.]
- (18) MOTTIER, D. M.
1918. CHONDRIOSOMES AND THE PRIMORDIA OF CHLOROPLASTS AND LEUCOPLASTS. *Ann. Bot.* [London] 32: [91]-114, illus.
- (18a) NAGELI, (K.)
1862. DIE REACTION VON JOD AUF STÄRKEKÖRNER UND "ZELLMEMBRANEN." *Sitzber. K. Akad. Wiss. München jahrg.*, 1862 (Bd. 2): 280-312.
- (19) PRÁT, S.
1927. THE TOXICITY OF TISSUE JUICES FOR CELLS OF THE TISSUE. *Amer. Jour. Bot.* 14: 120-125.
- (20) PRIESTLEY, J. H., and IRVING, A. A.
1907. THE STRUCTURE OF THE CHLOROPLAST CONSIDERED IN RELATION TO ITS FUNCTION. *Ann. Bot.* [London] 21: 407-413, illus.
- (21) RANDOLPH, L. F.
1922. CYTOLOGY OF CHLOROPHYLL TYPES OF MAIZE. *Bot. Gaz.* 73: 337-375, illus.
- (22) RUDOLPH, E.
1912. DAS CHONDRIOME DER PFLANZENZELLE. *Sitzber. "Lotos"* 70(7): 197-199. [Reviewed by Matouschek in *Bot. Centbl.* 122: 274-275. 1913.]
- (23) ————
1912. CHONDRIOSOMEN UND CHROMATOPHOREN. BEITRAG ZUR KRITIK DER CHONDRIOSOMEN THEORIE. *Ber. Deut. Bot. Gesell.* 30: 605-629, illus.
- (24) SACHS, J.
1875. TEXT-BOOK OF BOTANY, MORPHOLOGICAL AND PHYSIOLOGICAL. Transl. and annotated by A. W. Bennett, assisted by W. W. T. Dyer. 858 p., illus. Oxford.
- (25) SCHIMPER, A. F. W.
1882. ÜBER DIE GESTALTEN DER STÄRKEBILDNER UND FARBKÖRPER. *Bot. Centbl.* 12: 175-178.
- (26) ————
1883. ÜBER DIE ENTWICKLUNG DER CHLOROPHYLLKÖRNER UND FARBKÖRPER. *Bot. Ztg.* 41: [105]-112, [121]-131, [137]-146, [153]-162, illus.

-
- (27) ———
1885. UNTERSUCHUNGEN ÜBER DIE CHLOROPHYLLKÖRPER UND DIE IHNEN
HOMOLOGEN GEBILDE. *Jahrb. Wiss. Bot.* 16 : 1-246, illus. [Original not seen.]
- (28) SCHMITZ, F.
1882. DIE CHROMATOPHOREN DER ALGEN. 176 p. Bonn.
- (29) WIESNER, J.
1877. UEBER DAS VORKOMMEN UND DIE ENTSTEHUNG VON ETIOLIN UND
CHLOROPHYLL IN DER KARTOFFEL. *Osterr. Bot. Ztschr.* 27 : 7-11.
- (30) ZIRKLE, C.
1927. THE GROWTH AND DEVELOPMENT OF PLASTIDS IN LUNULARIA VUL-
GARIS, ELODEA CANADENSIS AND ZEA MAYS. *Amer. Jour. Bot.* 14:
429-445, illus.
- (31) ———
1929. DEVELOPMENT OF NORMAL AND DIVERGENT PLASTID TYPES IN ZEA
MAYS. *Bot. Gaz.* 88 : 186-203, illus.

SOME NEMIC PARASITES AND ASSOCIATES OF THE MOUNTAIN PINE BEETLE (*DENDROCTONUS MONTICOLAE*)¹

By G. STEINER²

Senior Nematologist, Division of Nematology, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

A study of the nemic parasites and associates of bark beetles is of both economic and scientific interest. Some of the new species proposed by Fuchs^{3 4} in his recent works on the nemic fauna of the bark beetles of central Europe must be considered detrimental to the beetles and therefore of economic importance. The limited observations recorded in the present paper indicate that the nemic fauna of all bark beetles known to occur in the United States should be investigated, as some of the nemas may prove of value in the control of the beetles by acting as disease carriers and as consumers of weak or even healthy beetles and their eggs.

Closely related to the determination of economic importance is the study of the ecological relations existing between the nemas and the beetles. Of special interest also is the classification of the various genera and species. Both of these latter aspects of the subject are discussed in the present paper.

MATERIAL

The material used in the present investigation was collected at Metaline Falls, Wash., in July and August, 1930. Some of it was taken from under the bark of a pine that had been killed by the mountain pine beetle (*Dendroctonus monticolae* Hopk.) in 1929 and some from a pine that had become infested not more than 10 days before the date of collection, July 18, 1930.

ECOLOGY

RELATION OF NEMAS TO BARK BEETLES

Some of the new nemic genera proposed by Fuchs⁵ are extremely close to free-living genera, and the various species represent almost every intermediate stage, thus illustrating the manner in which parasitic nemas may arise from free-living forms. From a mere occurrence of the nemas in the mines of the bark beetles and their possibly accidental use of the beetles as carriers, the way to true parasitism seems to proceed by the following steps: (1) The use of the bark beetle as unconditional carrier, (2) the extension of the carrier association into a regular life association under the wing covers or on

¹ Received for publication Jan. 21, 1932; issued October, 1932.

² The material for this investigation was collected by D. DeLeon, formerly of the forest insect field station, Bureau of Entomology, U. S. Department of Agriculture, at Coeur D'Alene, Idaho.

³ FUCHS, G. DIE NATURGESCHICHTE DER NEMATODEN UND EINIGER ANDERER PARASITEN. 1. DES IPS TYPOGRAPHUS L. 2. DES HYLOBIUS ABIETIS L. Zool. Jahrb., Abt. System., Geogr. u. Biol. Tiere 38 : [109]-222, illus. 1915.

⁴ ———. NEUE AN BORKEN UND RÜSSELKÄFER GEBUNDENE NEMATODEN, HALBPARASITISCHE UND WOHNUNGSEINMIETER. FREILEBENDE NEMATODEN AUS MOOS UND WALDERDE IN BORKEN-UND RÜSSELKÄFERGÄNGEN. Zool. Jahrb., Abt. System., Ökol. u. Geogr. Tiere 59 : [505]-646, illus. 1930.

⁵ FUCHS, G. Op. cit. (See footnote 3.)

the exterior surface of the beetle, (3) the production of special cocoons and webs by the nemas on the body of the beetle, (4) the habit of feeding on the beetle itself from the outside (ectoparasitism), and (5) true endoparasitism of the nemic larvae.

ECOLOGICAL GROUPS OF APHELENCHOIDES

Certain species of *Aphelenchoides*⁶ are free-living; others are parasitic, either on plants or animals; and there are numerous intermediate species. Fuchs separates his *Parasitaphelenchus* into the three following ecological groups:

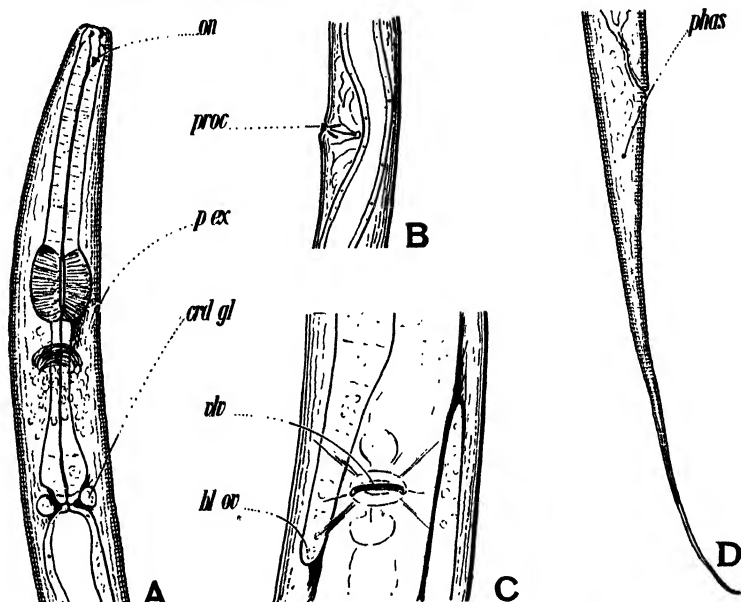


FIGURE 1.—*Diplogaster occidentalis* (female). A.—Head end in lateral view: *on*, onchium; *p ex*, excretory pore; *crd gl*, cardiac gland. \times about 750. B.—Vulvar region in lateral view: *proc*, toothlike process. \times about 530. C.—Vulvar region in ventral view: *vb*, vulva; *bl ov*, blind end of anterior ovary. \times about 1,000. D.—Tail: *phas*, phasmid. \times about 530

- (1) Species whose larvae live as parasites in the body cavity of bark beetles. (Endoparasites.)
- (2) Species whose larvae are found with or without a cocoon either under the wing covers of bark beetles or attached sucking on the backs of the beetles. (Ectoparasites.)
- (3) Species whose larvae and adults are found in the frass of the mines or on the beetles themselves, but under conditions not indicative of true parasitism. (Free-living.)

This classification outlines the three main aspects of the relationship between the species of *Aphelenchoides* and the bark beetles. The two species described in a later section of the present paper, from the mountain pine beetle of Washington, apparently represent true endoparasites. Only adults were seen, but it is highly probable that the larvae develop within the body cavity of the beetles. A still more pronounced type of parasitism is possible—that in which not only the larvae but also the adults are endoparasites. That this type of

⁶Synonyms: *Pathoaphelenchus* Cobb, and *Parasitaphelenchus* Fuchs. (See footnotes 4 and 10.)

parasitism occurs in the genus *Aphelenchoides* is not known, but it may be considered possible in view of the present meager knowledge of these forms.

A number of other genera described by Fuchs⁷ from various bark beetles show a trend toward parasitic life, but the process is especially impressive in *Aphelenchoides* because the most closely related forms, such as species of *Aphelenchus*, *Paraphelenchus*, and *Schistonchus*, are well-known free-living or plant-parasitic species. A somewhat new conception of the status of these genera is suggested in the present paper.

DIPLOGASTER AND THE MOUNTAIN PINE BEETLE

Tendencies toward parasitism are evident in the genus *Diplogaster*, although at present there is no species known that may properly be called a parasite. The species described herein is considered by the writer to be an associate, rather than a parasite, of the mountain pine beetle. These nemas inhabit the mines and frass and are probably carried by the beetles from one mine, tree, or locality to another.

TAXONOMY

DIPLOGASTER OCCIDENTALIS, N. SP.

Diplogaster occidentalis (figs. 1-3) is closely related to a group of forms, namely, *D. rhodani* Stefanski, *D. nudicapitatus* Steiner, *D. lineatus* Fuchs, and *D. consobrinus* De Man.⁸ Of these four forms, only *D. lineatus* is described from bark beetles. It was found in the mines of *Ilylobius abietis* L. from Europe. *D. occidentalis* is also found living in bark-beetle mines. In all, 175 specimens were observed, including 47 males, 92 females, and 36 larvae.

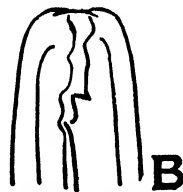
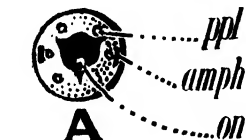


FIGURE 2.—*Diplogaster occidentalis*. A.—Front view of head: ppl, papilla; amph, amphid; on, onchium. \times about 1,610. B.—Free-hand sketch of pharynx

The largest males are about one and one-half times as large as the smallest. Males, females, and larval specimens have an elongated filiform tail; in the male the base is somewhat set off from the slender end portion. (Fig. 3, C.) The cuticle has 18 equidistant uninterrupted longitudinal wings of the same height. (Fig. 3, B.) The intermediate portions of the cuticle, however, show annulation. A cross section shows the lateral and ventral cords to be rather wide (fig. 3, B); no dorsal cord was seen. The head structures are somewhat obscure. There seem to be six lips, each having a papilla. (Fig. 2, A.) In a front view, small slits just behind the lateral papillae are interpreted as being the external amphids. The oral opening is triangular in shape, with the three corners well cuticularized and distinct. The form of the pharynx is rather indistinct; its walls are not well cuticularized and are only partly thickened (fig. 2, B); it is narrow but deep, and of somewhat irregular shape. What appears to be an onchium is situated at its base; for an onchium, however, it is little cuticularized. The anterior cylindrical portion of the esophagus is followed by a well-developed medial bulb, which exhibits numerous radial muscle fibers and long but thin valvulae. The isthmus and terminal bulb are typically diplogastroid, clear and rather transparent. Three cardiac glands were seen. The excretory pore opens ventrad of the middle bulb. The female apparatus is amphidelphic, with the reflexed ovaries extending back to the vulva or even past it. (Fig. 1, C.) The transparent vulva is slitlike (fig. 1, C); its posterior lip, in profile view, seems to form a toothlike process (fig. 1, B). The male has a single reflexed testis. (Fig. 3, A.)

⁷ FUCHS, G. Op. cit. (See footnote 4.)

⁸ MAN, J. G. DE. DESCRIPTION D'UNE ESPÈCE DU GENRE DIPLOGASTER MAX SCHULTZE: DIPLOGASTER CONSOBRINUS NOV. SP. Ann. Soc. Roy. Zool. Malacol. Belg. 31: 47-54, illus. 1920.

Its copulatory apparatus is remarkable because of the large-sized gubernaculum—nearly as long as the spicula—especially well seen in side view. Linear distally, it widens considerably toward its inner end, which forms a rather wide plate ending with straight transverse truncation. The spicula are much finer; their proximal ends distinctly capitate. Distally the gubernaculum exhibits in side view a larger and, proximad to it, a smaller process; the ventral view, however, shows only the larger process, which then appears very prominent. (Fig. 3, C.) The protrusile muscle of the gubernaculum is remarkably strong; oblique copulatory papillae occur in front of the anus. The arrangement of the male copulatory papillae is shown in Figure 3, C and D. Of special interest are the

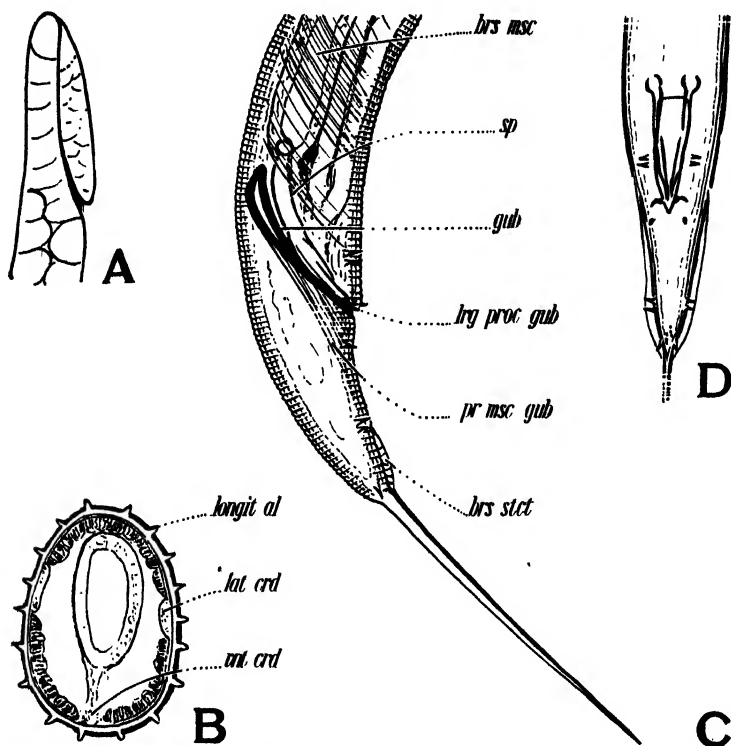


FIGURE 3.—*Iphopaster occidentalis* (male). A.—End of testis (free-hand sketch). B.—Cross section in middle region of body anterior to testis: *longit al*, longitudinal wing; *lat crd*, lateral cord; *vnt crd*, ventral cord. \times about 1,070. C.—Tail end: *brs msc*, bursal muscles; *sp*, spicula; *gub*, gubernaculum; *lrg proc gub*, larger process of the gubernaculum; *pr msc gub*, protrusile muscle of the gubernaculum; *brs stct*, bursalike structure. \times about 1,070. D.—Anal region in ventral view. \times about 665

small winglike or bursalike formations on each side of the tail of the male. (Fig. 3, D.) One of the papillalike lateral structures apparently connected with this wing may be a phasmid, which is clearly seen on the female tail. (Fig. 1, D.)

MEASUREMENTS:

Male:	1.1	13.0	19.0	'M 81.0	0.60 mm.
	1.9	4.5	4.8	5.6 3.7	
Male:	1.1	13.0	19.0	'M 80.0	0.43 mm.
	1.5	3.9	3.9	3.9 3.2	
Female:	0.7	13.0	17.0	'48,' 76.0	0.64 mm.
	1.3	3.0	3.5	4.1 2.8	
Female:	0.72	14.0	18.0	'46,' 75.0	0.625 mm.
	1.40	3.6	3.6	4.2 2.2	

DIAGNOSIS.—*Diplogaster* with 18 equidistant longitudinal wings, with distinct annulation in the interspaces. Head with six lips, each of which has a single papilla. Pharynx narrow, irregular, much longer than wide, without distinct separation into wider anterior and a narrow posterior portion, and with an indistinct, little cuticularized dorsal onchium at its base. Middle esophageal bulb with long but thin valvulae. Female apparatus amphidelphic, ovaries reflexed, ending past the vulva; posterior vulvar lip in profile view with small toothlike structure. Male and female tail long, conical at base, filiform at end portion. Male with a very prominent gubernaculum, at least five-sixths of the length of the spicula; copulatory papillae as shown in Figure 3, C and D.

THE GENUS APHELENCHOIDES

STATUS OF THE GENUS

Two species from the material used in the present study were found to be very closely related to a number of species recently described by Fuchs⁹ under the generic name of *Parasitaphelenchus*. Unfortunately Fuchs failed to give a generic diagnosis. His description reveals no characters that apply to all his species or differentiate them from other genera such as *Aphelenchoides*. All his species and also the two new species described in the present paper may properly be placed in the latter genus. The generic name *Aphelenchoides* is here used in the sense suggested by Cobb for *Pathoapphelenchus*¹⁰ (which is antedated by the name *Aphelenchoides*¹¹). Its characters, which are here repeated, are those of the former genus *Aphelenchus*, but not those that must be recognized since the discovery of the males of the type species of *Aphelenchus*, *A. avenae*. This species exhibits characters that separate it widely from the forms with which it has hitherto been associated and which must now be included in the genus *Aphelenchoides*. The writer^{12 13} has given a fuller discussion of this question elsewhere.

Fuchs¹⁴ mentions for four of his *Parasitaphelenchus* species a character which, if proved to be valid, would fully justify the creation of a new genus, namely, the location of the nerve ring in front of the esophageal bulb in the species *Parasitaphelenchus typographi*, *P. dubius*, *P. conjunctus*, and *P. hylastophilus*. This position of the nerve ring, if established, is exceedingly remarkable, so much so that further observations must be made before it can be accepted. If, however, it is accepted as a generic character, most of the species included by Fuchs in the genus *Parasitaphelenchus* would have to be excluded.

At present, therefore, it is not possible to recognize *Parasitaphelenchus* as a genus in good standing, but it should be synonymized with *Aphelenchoides*. If a division of the latter genus is to be made, it could only be on characters that are not yet entirely clear and certain. If the future should show the need for the creation of a new genus, its characters will have to be formulated anew.

⁹ FUCHS, G. Op. cit. (See footnote 4.)

¹⁰ COBB, N. A. [APHELENCHUS RETUSUS WITH A PROPOSED DIVISION OF APHELENCHUS.] Helminthol. Soc. Wash. Proc. Jour. Parasitol. 14: 57. 1927.

¹¹ FISCHER, M. ÜBER EINE CLEMATIS-KRANKHEIT. Ber. Physiol. Lab. Landw. Inst. Halle 3 (Heft 11): 1-11, illus. 1894.

¹² STEINER, G. ON THE STATUS OF THE NEMIC GENERA APHELENCHUS RASTIAN, PATHOAPHELENCHUS COBB, PARAPHELENCHUS MICOLETEKY, PARASITAPHELENCHUS FUCHS, ISONCHUS COBB AND SEINURA FUCHS. Jour. Wash. Acad. Sci. 21: 468-476, illus. 1931.

¹³ ———. ANNOTATIONS ON THE NOMENCLATURE OF SOME PLANT PARASITIC NEMATODES. Jour. Wash. Acad. Sci. (In press.)

¹⁴ FUCHS, G. Op. cit. (See footnote 4.)

The generic characters of *Aphelenchoides* are as follows:

(1) The males have no bursa but only copulatory papillae; the spicula are short, strongly arcuate, proximally wide to very wide, distally more or less pointed; the gubernacula are absent; the testis is single.

(2) The postbulbar portion of the esophagus is more or less distinctly assimilated by the intestine, which therefore appears to begin immediately behind the esophageal bulb. This latter is the homologue of the middle esophageal bulb in *Tylenchus* and other genera.

(3) The outlets of the three salivary (esophageal) glands are located in the esophageal bulb, the dorsal one anterior and the two ventro-submedial ones posterior to the valvulae.

(4) The females are prodelphic with a more or less reduced posterior gonadic branch still present.

APHELENCHOIDES CONURUS, N. SP.

Only two specimens of *Aphelenchoides conurus* (fig. 4) were seen, and unfortunately one of them was lost during preparation. The following description is therefore based mainly upon a single female.

The tail is elongate-conical, but, in contrast to other species, not mucronate. The cuticle is annulated but has no wings. The specimen observed had a distinct lip region which exhibited all the characters typical of the genus. There are four submedial papillae, and laterally rather distinct amphidial openings. The spear is short but rather wide; notable is the absence of knots or swellings at its proximal end. The spear thus offers the best specific characters. The esophageal bulb is exceptionally large (fig. 4, A); its valvulae are distinct but not prominent; radial muscle fibers were not seen. The ampullae and outlets of the salivary or esophageal glands could be distinguished; they seem to have the normal position, that is, the position typical for *Aphelenchoides*. The dorsal gland opens in the anterior portion of the bulb and the two subventral ones in the posterior. The intestine begins at the bulb as a cylindrical tube which, however, swells after it has passed through the nerve ring. Its lumen is very indistinct; its tissue is filled with reserve material, the cell walls being therefore obscure. The rectum, too, is rather indistinct, perhaps not even

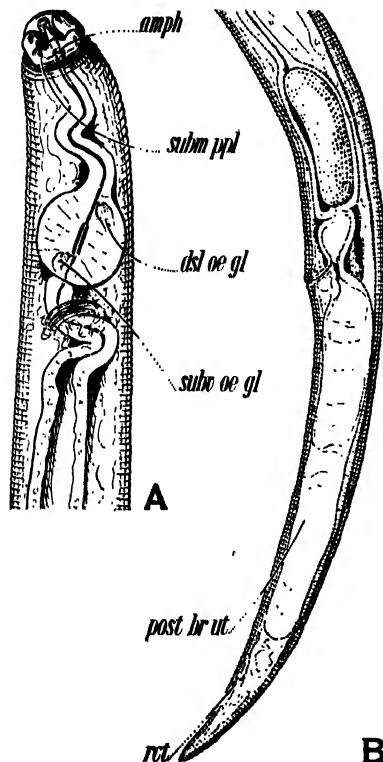


FIGURE 4.—Parts of *Aphelenchoides conurus*. A.—Head end of female: *amph*, amphid; *subm ppt*, submedial papilla; *dsl oe gl*, outlet of dorsal esophageal gland; *subv oe gl*, outlet of subventral esophageal gland. \times about 530. B.—Posterior portion of female: *post br ut*, posterior branch of uterus; *rect*, indistinct rectum. \times about 360

functional. The excretory pore was not seen. The female apparatus is prodelphic, but, as in other members of the genus, a large posterior branch of the uterus extends nearly to the anus. The vagina leads slightly forward. The eggs are about three times as long as wide.

MEASUREMENTS:

	0.8	6.3	7.0	80.0	96.3	
Female:	1.1	2.3	2.4	2.8	1.3	1.0 mm.

DIAGNOSIS—*Aphelenchoides* with elongated, conical nonmucronate tail. Spear short but wide, without basal swellings. Esophageal bulb large, spherical, apparently without radial fibers. Living as parasite and associate of the mountain pine beetle.

APHELENCHOIDES ACROPOSTHION, N. SP.

Several females and males of *Aphelenchoides acroposthion* were found. Compared with other members of the genus, this species is a very large form.

Although the specimens had the appearance of true parasites, no exact facts were known as to their relationship to the mountain pine beetle except that they have been found in the mines of the beetles. Since only adult specimens were present, one is led to suppose that their larvae may be endoparasites of the beetle.

The body is very slender and elongate. The tail of the female is short and bluntly rounded (fig. 5, A); that of the male is also short, and its base is similar to that of the female but with the difference that it ends in a short hornlike process (fig. 5, D). The cuticle is finely annulated, but no lateral membrane was seen. The distinctly set-off lip region exhibits in a front view six rounded, well-separated lips. (Fig. 5, B.) A cuticularized ring with six short rays encircles the oral opening. The structures of the head end were rather obscure, but it is believed that the amphids were seen in the position shown in Figure 5, B. This species, like other members of the genus, has four submedial papillae. The spear is extremely fine (fig. 5, E) and appears to be composed of three different portions—an anterior conical portion rather well cuticularized, a succeeding short cylindrical portion also well cuticularized, and then a long cylindrical portion which is hardly cuticularized and which, posteriorly, is set off from the esophageal canal only by the attachment of the protruding spear muscles. No basal swellings were seen. The esophageal bulb is well developed and of oval shape; it has rather long but thin valvulae. (Fig. 5, F.) The radial muscles attached to the valvulae exclude a more glandular portion at the anterior and posterior ends of the bulb. The connection of the intestine with this bulb is much the same as in other forms of *Aphelenchoides*. The nerve ring occurs a short distance behind the bulb. The intestine is of somewhat degenerate character; its cell walls can hardly be recognized, the whole organ being filled with reserve material. As in the previous species, the rectum and anal opening are extremely fine and obscure. No excretory pore was seen. The vulvar opening is well marked because the body narrows just behind it. The female sexual apparatus is prodelphic; there is, however, a well-developed posterior branch of the uterus, and attached to it there appears to be a vestigial ovary extending nearly to the anus. The anterior ovary extends forward nearly to the nerve ring. The very short sexual apparatus of the male is of the pro-orchid type, with the end of the testis reflexed. Figure 5, D, gives a lateral view, and Figure 5, C, a ventral view of the copulatory apparatus. It seems that the two spicula are amalgamated, forming a single spiculum, pointed at the outer end, but very wide at the inner end. The ventral apophysis exhibits at its inner end a short process pointing ventrad; the dorsal apophysis is somewhat swollen proximally. Only one male was studied in ventral view; it showed in the anal region a peculiar lateral expansion somewhat resembling a vestigial bursa. Two papillae were distinctly seen on the inside of this expansion, one in the anal region and the other at the base of the hornlike process. It is doubtful whether these males are fully functional.

MEASUREMENTS:

	0.8	5.7	6.2	M	99.2	
Male:		0.9	0.9	1.6	0.6	1.6 mm.
	0.5	3.5	4.1	78.2	3 ¹⁶	92.0
Female:	0.4	0.8	0.8		1.2	0.9
						2.4 mm.

DIAGNOSIS.—*Aphelenchoides* of long, slender shape. Tail of female short, obtusely rounded; that of male broadly conical at the base, mucronate. Spear rather narrow, of average length, without basal swellings. Esophageal bulb ovoid. Vulva well marked because the body narrows abruptly behind it. Male with slightly sublateral papilla in latitude of the anus, and another somewhat in front of terminal process.

SUMMARY

Some nemic associates and parasites of the mountain pine beetle are described and their economic significance is discussed. The various phases of the mutual relationship of the bark beetle and the

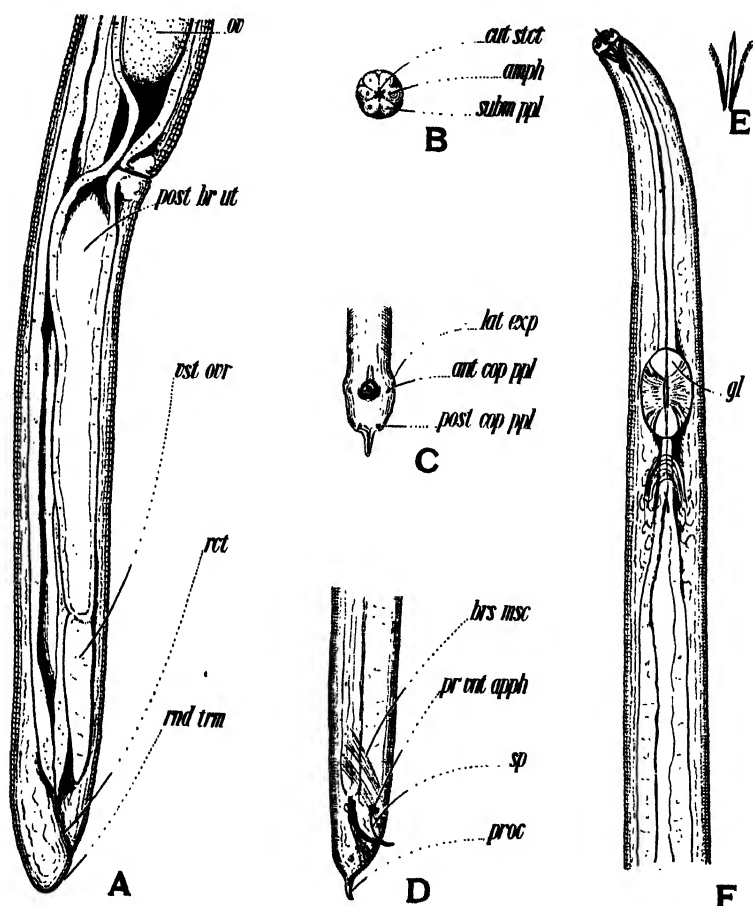


FIGURE 5.—*Aphelenchoides acroposthion*. A.—Posterior portion of female: *ov*, egg; *post br ut*, posterior branch of uterus; *rst ovr*, vestigial ovary; *rct*, rectum; *rnd trm*, rounded tail. \times about 720. B.—Front view of head: *cut sclt*, cuticularized structure; *amph*, amphid; *subm ppl*, submedial papilla. \times about 940. C.—Tail of male, ventral view: *lat exp*, lateral expansion; *ant cop ppl*, anterior copulatory papilla; *post cop ppl*, posterior copulatory papilla. \times about 720. D.—Tail of male, lateral view: *brs msc*, bursal muscles; *pr ant apph*, process of ventral apophysis; *sp*, spiculum; *proc*, hornlike process (muera). \times about 720. E.—Free-hand sketch of spear. F.—Anterior portion of body: *gl*, granulated portion of esophageal bulb. \times about 720.

nemas are considered (free association, carrier relationship, ectoparasites, endoparasites). The taxonomy of the genus *Aphelenchoides* and three nemas new to science, *Diplogaster occidentalis*, *Aphelenchoides conurus*, and *A. acroposthion*, are discussed and morphological descriptions given.

THE VITAMIN A, B, C, AND G CONTENT OF CONCORD GRAPES¹

By ESTHER PETERSON DANIEL, *Associate Nutrition Chemist*, and HAZEL E. MUNSELL, *Senior Nutrition Chemist in Charge, Nutrition Studies Section, Foods and Nutrition Division, Bureau of Home Economics, United States Department of Agriculture*

INTRODUCTION

During an earlier study² the vitamin A, B, C, and G content of Sultanina (Thompson Seedless) and Malaga grapes as well as two brands of commercial grape juice was determined. Since the completion of this work, there have been numerous requests for information concerning the vitamin content of Concord grapes (*Vitis labrusca*), and in consequence the study presented here was conducted.

PLAN OF STUDY .

The Concord grapes tested were purchased at retail stores and were therefore representative of the fruit available to the consumer. Only the edible portion of the fruit was used in the experiments. This portion consisted of as much pulp and juice as could be removed from the skins, which were discarded as nonedible. The edible portion of the fruit was fed in 2, 4, and 6 g daily amounts in the tests for the vitamin A, B, and G content, and in 10, 12, and 15 g daily allotments in the vitamin C determination. Eight rats were fed each dose tested for vitamin A, B, and G, and seven guinea pigs were used in the vitamin C determinations.

Following the technic used in the earlier studies, vitamin A was determined by the Sherman and Munsell method,³ vitamin B by a method worked out in this laboratory similar to one outlined by Chase,⁴ vitamin C by the histological procedure described by Höjer,⁵ and vitamin G by the writers' own method, which is not unlike that used by Sandels.⁶ The diets and procedures used in determining each of these vitamins are given in the earlier paper.⁷

RESULTS

A summary of the data obtained in the vitamin A determination, illustrated in Figure 1, shows that 6 g of the edible portion of Concord grapes fed as the sole source of vitamin A induced but very little growth in the rats during the 8-week test period. From this evidence

¹ Received for publication June 15, 1932; issued October, 1932.

² DANIEL, E. P. and MUNSELL, H. E. THE VITAMIN A, B, C, AND G CONTENT OF SULTANINA (THOMPSON SEEDLESS) AND MALAGA GRAPES AND TWO BRANDS OF COMMERCIAL GRAPE JUICE. *Jour. Agr. Research* 44: 59-70, illus. 1932.

³ SHERMAN, H. C., and MUNSELL, H. E. THE QUANTITATIVE DETERMINATION OF VITAMIN A. *Jour. Amer. Chem. Soc.* 47: 1639-1646, illus. 1925.

⁴ CHASE, E. F. A QUANTITATIVE STUDY OF THE DETERMINATION OF THE ANTI-NEURITIC VITAMIN (F OR B). 41 p., illus. New York, 1928. (Thesis, Ph. D., Columbia Univ.)

⁵ HÖJER, J. A. STUDIES IN SCURVY. *Acta Paediatrica* 3; Supplementum, 278 p., illus. Uppsala. 1924. METHOD FOR DETERMINING THE ANTISCORBUTIC VALUE OF A FOODSTUFF BY MEANS OF HISTOLOGICAL EXAMINATION OF THE TEETH OF YOUNG GUINEA-PIGS. *Brit. Jour. Exptl. Path.* 7: 356-360, illus. 1926.

⁶ SANDELS, M. R. RELATIVE SOLUBILITIES OF THE ANTINEURITIC AND ANTIPELLAGRIC VITAMINES IN ALCOHOL, AND A STUDY OF CERTAIN PROPERTIES OF THESE SUBSTANCES. 71 p., illus. New York. 1928. (Thesis, Ph. D., Columbia Univ.)

⁷ DANIEL, E. P., and MUNSELL, H. E. *Op. cit.*

it must be concluded that the pulp and juice portion of the Concord grape is a poor source of vitamin A. The edible portions of the Sultanina (whole) and Malaga (seeded) grapes, which contain only small amounts of vitamin A, as shown by the earlier investigation, are nevertheless a richer source of this vitamin than the edible portion of Concord grapes. This is evident from the fact that 5 g daily of

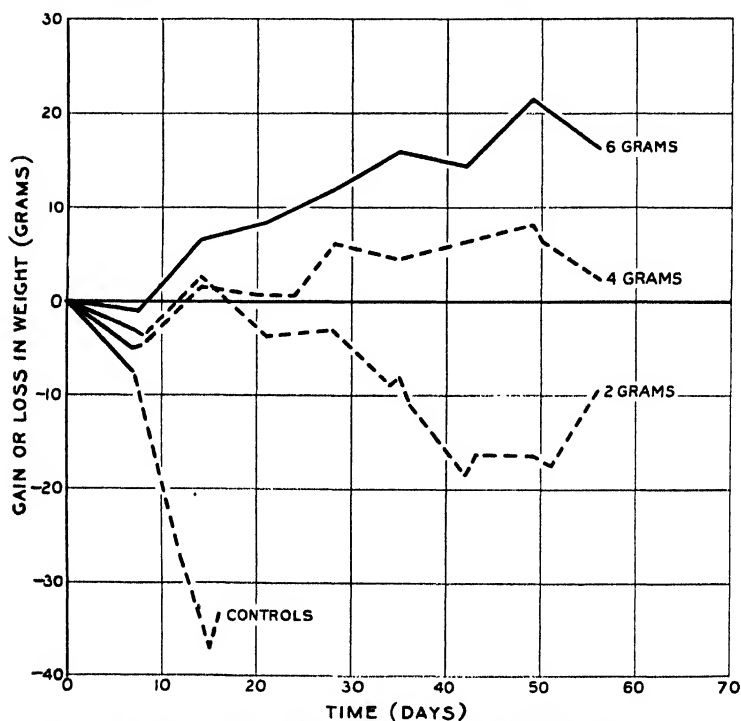


FIGURE 1.—Average gain or loss in weight of groups of young rats fed the edible portion of Concord grapes (*Vitis labrusca*) as the sole source of vitamin A. The broken lines begin at a point indicating the occurrence of the first death in the group. The weighed quantity of grapes fed daily six times a week is indicated at the end of the curves

Sultanina or Malaga grapes produced a greater gain in weight than did 6 g daily of Concord grapes.

The results of the vitamin B tests, portrayed graphically in Figure 2, show that there are very small but detectable amounts of vitamin B in the edible portion of Concord grapes. In contrast to these findings, Malaga (seeded) and Sultanina (whole) grapes proved to be fair sources of this vitamin.

Although 10, 12, and 15 g quantities of the Concord grape pulp and juice were fed daily as a source of vitamin C, the results of a histological examination of the teeth of the experimental guinea pigs showed no evidence of protection. The same quantity of Malaga (seeded) and Sultanina grapes (whole), while insufficient to give complete protection, contained small amounts of vitamin C.

There is no demonstrable amount of vitamin G in the edible portion of Concord grapes. (Fig. 3.) Malaga (seeded) grapes also

showed no evidence of the presence of this vitamin, while Sultanina (whole) grapes contained only a very slight quantity.

The edible portions of the Malaga (seeded) and Sultanina (whole) grapes were, with one exception, richer in each of the vitamins tested

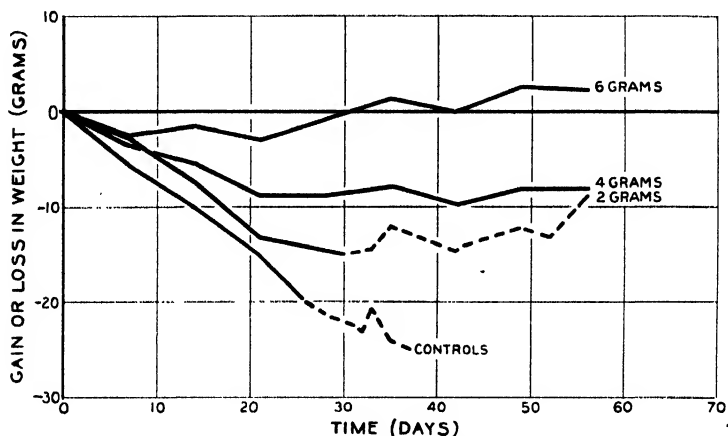


FIGURE 2.—Average gain or loss in weight of groups of young rats fed the edible portion of Concord grapes (*Vitis labrusca*) as the sole source of vitamin B (antineuritic). The broken lines begin at a point indicating the occurrence of the first death in the group. The weighed quantity of grapes fed daily six times a week is indicated at the end of the curves

than was the edible part of the Concord grapes; the Malaga and Concord grapes were both lacking in vitamin G. It may be possible that some of the vitamin value of the Concord grapes was lost when

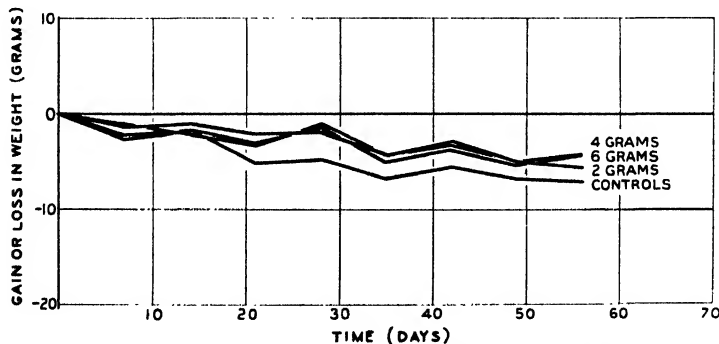


FIGURE 3.—Average gain or loss in weight of groups of young rats fed the edible portion of Concord grapes (*Vitis labrusca*) as the sole source of vitamin G. The weighed quantity of grapes fed daily six times a week is indicated at the end of the curves

the skins were discarded. The skins were retained in the tests made on Malaga and Sultanina grapes. It is known that in the case of some other fruits vitamins tend to be concentrated in the skin or in the portion directly beneath the skin.

SUMMARY

The vitamin A, B, C, and G content of the edible portion of Concord grapes (*Vitis labrusca*) was determined. The edible portion was considered as the part remaining after the removal of the skins.

Results showed that this portion of Concord grapes contains a very small amount of vitamin A and barely detectable amounts of vitamin B. There was no evidence of the presence of either vitamin C or vitamin G.

Earlier studies indicate that edible portions of Malaga (seeded) and Sultanina (whole) grapes (*Vitis vinifera*) possess a greater vitamin A, B, and C content than does the edible portion of Concord grapes. Sultanina grapes appear to contain a small amount of vitamin G, which makes them a richer source of this vitamin. One explanation for these differences may be found in a possibly greater concentration of vitamin in the skins, which were retained in the case of the Sultanina and Malaga grapes and were discarded as a nonedible portion of the Concord grapes.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 45

WASHINGTON, D. C., OCTOBER 15, 1932

No. 8

EFFECT OF CARBON DIOXIDE CONTENT OF STORAGE ATMOSPHERE ON CARBOHYDRATE TRANSFORMATION IN CERTAIN FRUITS AND VEGETABLES¹

By ERSTON V. MILLER, *Assistant Physiologist*, and CHARLES BROOKS, *Principal Pathologist, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*²

INTRODUCTION

In an earlier article the writers (7)³ have discussed the inhibiting effect of carbon dioxide on the growth of fruit-rotting fungi and its retarding effect on the ripening processes of fruits and vegetables in storage. The present paper supplements the previous one by presenting the results of chemical analyses of fruits and vegetables treated with carbon dioxide.

WORK OF OTHER INVESTIGATORS

Many investigators have studied the chemical changes that take place in fruits and vegetables after harvest and during storage. Boswell (5) has shown that in the ripening processes of pea seeds there occurs a rapid decrease in the percentage of sugar and soluble nitrogenous substances and an increase in starch, total acid-hydrolyzable substances, and insoluble nitrogen compounds (largely proteins). He adds that these changes are so rapid that a slight delay in harvesting results in markedly low quality, and that high sugar and low starch content in young peas is essential for high quality.

Jones and Bisson (8a) found that at 0° C. no important changes occur in the carbohydrate content of freshly harvested peas. At temperatures above 0° there is a loss in percentage of the more mobile carbohydrates and an increase in percentage of the more stable carbohydrates. These changes appeared to occur most rapidly at 25°, and more rapidly in shelled than in unshelled peas.

Kertesz (8b) reported that changes in the chemical composition of green peas begin immediately after shelling. Important among these changes are loss of sucrose and increase in alcohol-insoluble residue.

A number of investigators have studied the rapid depletion of sugar in green sweet corn after its removal from the stalk. That sweet corn loses a large percentage of its sugar content after removal, especially at summer temperatures, has been demonstrated by Straughn (11), Straughn and Church (12), Stevens and Higgins (10), Appleman and Arthur (1), and others. Appleman and Arthur found that the depletion of sugar does not proceed at a uniform rate but is more rapid at first and slows down as it approaches equilibrium, which is reached at the point where about 62 per cent of the total sugar and about 70 per cent of the sucrose have been lost. They state that in general

¹ Received for publication Dec. 18, 1931; issued October, 1932.

² The writers are indebted to Howard B. Johnson, junior pathologist, and Oscar J. Dowd, junior physiologist, for assistance in performing the experiments recorded in this paper.

³ Reference is made by number (*Ref.*) to Literature Cited, p. 458.

the rate of loss is doubled for every 10 degrees' increase in temperature between 10° and 30° C. until it reaches 50 per cent of the initial total sugar and 60 per cent of sucrose.

Sweet cherries have been studied by Hartman and Bullis (8), who report that in ripening there is an increase in the sugar and solid content of the fruit, a decrease in acidity and astringency, and an increase in specific gravity of both the fruit and fruit juice. Except for loss of weight and volume, there were no striking chemical or physiological changes in the fruit immediately after removal from the tree.

Bigelow and Gore (4) conducted studies on the after-harvest changes in "market-ripe" peaches at three different storage temperatures. They divided the actual daily loss in grams of the substance per peach by the solid matter in the flesh at the beginning of storage. Averaging the results of several varieties, they found a loss of "marc," acids, and sucrose at each temperature and in general an increase of reducing sugars.

Appleman and Conrad (2), in studies of the pectic constituents of peaches, showed that the conversion of protopectin to pectin could be greatly retarded at low temperatures.

Kidd and West (9) reported that an atmosphere of 5 to 15 per cent O₂ combined with 10 to 15 per cent CO₂ about doubles the storage life of apples so long as the temperature is above that associated with low-temperature injury, and that the rate of loss of sugars was about halved under optimum gas-storage conditions as compared with storage in air at the same temperature.

Recently Thornton (13) reported on the tolerance of fruits, vegetables, and flowers to CO₂ and discussed the use of the gas for retarding the ripening of bananas and for removing the astringency of pears.

EXPERIMENTAL PROCEDURE

MATERIAL AND TREATMENT

In the present work the fruits and vegetables were treated with various percentages of CO₂ for certain periods of time. Comparable material was placed at each of the temperatures 0°, 5°, 10°, 15°, and 20° C. In some of the experiments a temperature range of 5°, 10°, 15°, 20°, and 25° was substituted. Constant temperatures were maintained by means of the chambers employed by Brooks and Cooley (6). There were two lots at each temperature, one receiving the CO₂ treatment and the other not treated and therefore considered as a control. The treated lots were placed in 9-liter glass jars, the ground-glass covers of which had been replaced by 2-hole metal covers sealed with plasticine. The controls were held in glass moist chambers or similar 9-liter glass jars without stoppers.

A constant percentage of carbon dioxide was maintained in the test chambers by means of a continuous renewal of the storage air. The desired concentration of CO₂ was obtained by mixing ordinary laboratory air with CO₂. By means of water displacement the gaseous mixture was driven through copper tubing into the experimental jars in the constant-temperature chambers. The gas was passed through coils of copper tubing within the chambers before entering the jars in order to bring it to the same temperature as the fruit in the jars. By means of a pinch clamp the rate of flow of the gas was so regulated as to permit about 90 gallons of gas to pass through in a period of 24

hours. At the beginning of each experiment the gas was first bubbled through very rapidly until the atmosphere surrounding the fruit had attained the desired concentration of CO_2 . The exact concentration of CO_2 employed in the experiment was determined by means of an Orsat gas apparatus, the percentage usually falling a little below the theoretical.

Peas, sweet corn, peaches, and cherries were used for the experiments. Alaska, Gradus, and Nott Excelsior peas were obtained from the Arlington Experiment Farm, Rosslyn, Va., near Washington, D. C. Sutton Perfection peas, grown at Mountain Lake Park, Md., were brought to Washington under "top icing." A recording thermometer in the pack indicated that the temperature was about 0°C . while the peas were in transit. Cherries and peaches were procured from the open market, the peaches being in what was apparently the "market ripe" stage of maturity. The sweet corn was obtained either from the Arlington farm or from Falls Church, Va., and was harvested early in the morning and quickly brought to the laboratory.

The amount of material employed for each lot was 250 g of peas in pod, 100 g of cherries, 10 to 15 peaches, and 4 half ears of corn in the "milk" stage. The ears of corn were cut in half transversely, and one half ear with husk was placed in the CO_2 lot and the other half in the control. Thus each lot contained the halves of four different ears. Alternate ears were reversed in order that each lot might receive first the tip half and then the butt half.

When the treatment extended over a period of from three to six days samples were removed every 24 or 48 hours. In these instances over a kilogram of peas and 10 ears of corn were employed for each lot.

SAMPLING

At the end of the CO_2 treatment the lots were removed, tested for flavor, and preserved for subsequent moisture and carbohydrate determinations.

For moisture samples, 3 to 5 g of macerated peas or corn and 5 to 10 g of peaches or cherries were placed in a weighing bottle, weighed, covered with 10 c c of absolute alcohol, and dried to constant weight at 70° to 80°C . in a vacuum equal to 25 inches of mercury.

The material for the carbohydrate analyses was preserved in alcohol. The peas were hulled and sampled whole, the cherries were pitted and sliced, the peaches were peeled, pitted, and ground in a food grinder, and two to four rows of sweet-corn kernels were completely removed from each ear by means of a sharp scalpel. The fresh material was dropped into a counterpoised Kohlrausch flask containing 0.25 g of CaCO_3 , weighed on a torsion balance, and covered with sufficient hot 95 per cent alcohol to make the final mixture 80 per cent. The samples were boiled on a water bath for from three to five minutes. Duplicate samples contained 20 g of peas, 40 g of cherries, 20 or 40 g of peaches, and 16 or 20 g of corn.

CHEMICAL ANALYSES

Sugar was extracted from the sweet-corn samples either by the method of Appleman and Arthur (1) or in accordance with the recommendations of the Association of Official Agricultural Chemists (3). When the extract was cooled to 20°C . the volume was made up to 200 c c, filtered, and 150 c c was withdrawn for analyses.

With peaches and cherries the supernatant alcohol was decanted off and the sample given three 30-minute refluxings with 80 per cent alcohol. Peas were extracted in the same manner, except that they were ground in a mortar before refluxing. The alcoholic extract of peas, cherries, and peaches was made up to a volume of 500 c c.

The alcoholic aliquots from all samples were evaporated down on a water bath, the alcohol being replaced with distilled water. When cooled again the aqueous solution was transferred to 200 c c flasks, clarified with neutral lead acetate, and the excess lead removed by means of sodium carbonate or potassium oxalate. The volume was made up to 200 c c, and reducing sugars were determined by the Munson and Walker method. Aliquots of 50 c c were reserved for total sugars. In some experiments the total sugar was determined as reducing sugar after inversion with hydrochloric acid and neutralization with sodium carbonate. In other experiments the inversion was made by a commercial preparation of invertase and reported as sucrose. In either case the results were computed as sucrose. Total sugar reported for peaches and sweet corn represents the sum of reducing sugar and sucrose. In peas only traces of reducing sugar were found, and in cherries only traces of sucrose.

The sugar-free residues were retained for determination of acid-hydrolyzable polysaccharides. (Completeness of sugar extraction was determined by the alpha-naphthol test.) The dried sample was placed in a Kjeldahl flask containing 200 c c of distilled water and 20 c c of dilute hydrochloric acid (specific gravity 1.125) and hydrolyzed for three hours in a water bath. The solution was neutralized with sodium carbonate, and reducing substances were determined by the Munson and Walker method. The results were computed as starch and reported as acid-hydrolyzable polysaccharides.

RESULTS

PEAS

The effect of the treatment with CO₂ on the carbohydrate content of Alaska and Sutton Perfection peas is shown in Table 1.

TABLE 1.--Total sugar and acid-hydrolyzable polysaccharides in peas treated with CO₂

[Expressed as percentage of fresh weight]

Variety	Treatment		Total sugar		Acid-hydrolyzable polysaccharides	
	CO ₂	Temperature (°C.) and time	In treated product	In control	In treated product	In control
Alaska	44	Per cent				
		(25°, 1 day	4.6	2.6	5.9	8.7
		20°, 1 day	4.3	3.0	6.5	8.3
		15°, 1 day	4.7	3.1	7.0	8.3
		10°, 2 days	4.2	3.1	6.7	8.1
		5°, 2 days	4.3	4.2	6.9	7.7
Sutton Perfection	42	20°, 1 day	5.8	4.9	2.9	4.2
		15°, 1 day	5.9	5.6	2.6	3.2
		10°, 1 day	6.0	5.7	3.0	3.3
		5°, 2 days	6.1	6.2	2.8	2.6
		10°, 2 days	6.4	6.6	3.7	3.8

With both varieties the CO_2 treatment resulted in the retention of a higher percentage of total sugar and a lower percentage of polysaccharides at all but the lower temperatures. The difference between the treated and control lots is much more pronounced with the Alaska peas than with the Sutton Perfection. A possible explanation of this may be found in the fact that in 1930 this section of the country experienced the worst drought on record, the effects of which were not so evident when the Alaska peas were harvested as later when the Sutton Perfection peas were ripe. In the case of the Alaska peas it will be observed that the treated lots at 10° , 15° , and 20° C. retained 35, 51, and 43 per cent more total sugar, respectively, than did the controls at these temperatures. At 25° there was 76 per cent more sugar in the treated lots than in the controls.

With the Sutton Perfection peas the treated lots at 10° and 15° each contained 5 per cent more sugar than the controls, and at 20° there was 18 per cent more sugar in the treated lots than in the controls.

The results for the Gradus and Nott Excelsior peas appear in Figures 1 and 2, respectively.

Both treated and control lots at 5° , 15° , and 25° were compared with a control at 0° C.

The excess of sucrose in the treated lots over the controls is most pronounced at 25° and 15° C. and is more marked as the time element increases. At the end of two days the treated Gradus peas at 15° contained over 10 per cent more sugar than did the controls at the end of the same period. During this time Nott Excelsior peas at 15° contained over 20 per cent more sugar than the controls. The contrast in the case of the Gradus peas was much more pronounced for the 3-day treatment. At the end of three days the flavor of the Nott Excelsior peas at 15° and 25° was objectionable. The low temperature of 5° appeared as effective as the CO_2 treatment in retaining the sucrose in the peas.

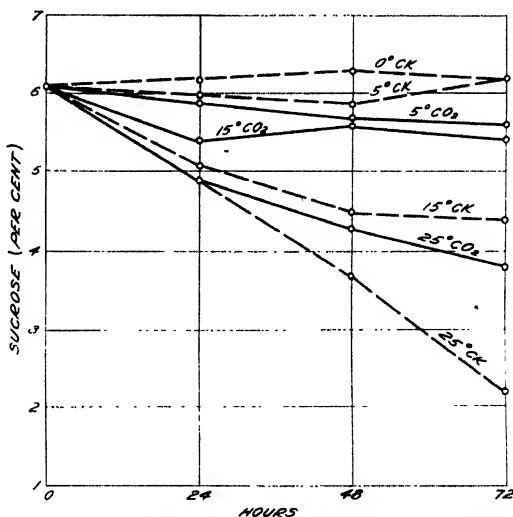


FIGURE 1.—Effect of exposure to 47 per cent CO_2 on sucrose in Nott Excelsior peas at various temperatures. (Solid lines, treated; broken lines, controls)

SWEET CORN

The results of the CO_2 treatment upon the sugar content of Stowell Evergreen sweet corn will be found in Table 2.

TABLE 2.—Total sugar in Stowell Evergreen sweet corn treated with CO₂

[Expressed as percentage of fresh weight]

Temperature (° C.) and time of treatment	35 per cent CO ₂		45 per cent CO ₂	
	Treated	Control	Treated	Control
20°, 1 day	3.7	3.1	4.1	3.1
15°, 1 day	3.3	3.2	4.3	3.7
10°, 1 day	3.8	3.4	4.2	3.7
5°, 2 days	4.5	4.2	4.3	4.1
0°, 2 days	4.8	5.2	5.3	4.6

It will be noted that with 45 per cent CO₂ the treated lots at all temperatures retained a higher percentage of total sugar than did the controls, the contrast being greater at the higher temperatures. The sweet corn treated at 20° C. contained about 30 per cent more sugar than the untreated lot at the same temperature, whereas at 15° and 10° the treated lots contained over 10 per cent more sugar than the untreated lots. The effect of 35 per cent CO₂ on sweet corn was similar to, though not so great as, that of 45 per cent CO₂.

The results for the Early Evergreen and Golden Bantam sweet corn appear in Figures 3 and 4, respectively.

In contrast to the results for peas, the effect of CO₂ on the sugar content of sweet corn was less pronounced at 25° C. than at the lower temperatures. This is especially true for Golden Bantam corn. The general effect of the CO₂ on both varieties was to retard the sugar loss during the storage

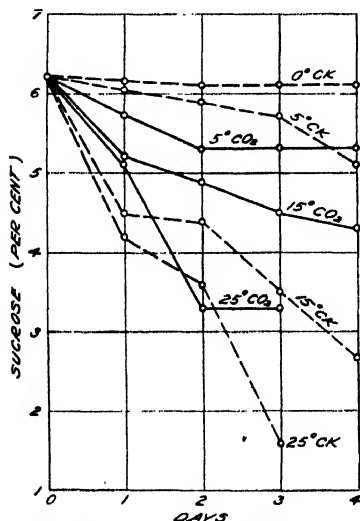


FIGURE 2.—Effect of exposure to 44 per cent CO₂ on sucrose in Gradus peas at various temperatures. (Solid lines, treated; broken lines, controls)

period. In the results for Early Evergreen sweet corn in Figure 3 it is interesting to note the grouping of the curves for the second day. Apparently the CO₂ treatment was equivalent to a 10-degree drop in temperature for the 25° and 15° lots and a 5-degree drop for the 5° lot.

In the Golden Bantam variety the CO₂ was ineffective in retarding the loss of sugar at 25° C. At 15°, however, there was over 20 per cent more total sugar in the treated than in the untreated lots for the first and second days. On the third day the sugar in the treated lot had dropped to the level of the control. At 5° the treated lots contained over 20 per cent more total sugar than the controls for two, four, and six days, but the corn had begun to lose its palatability on the sixth day.

PEACHES AND CHERRIES

In Table 3 will be found the results of CO_2 treatment of sour cherries, sweet cherries (Bing variety), and Belle peaches. The

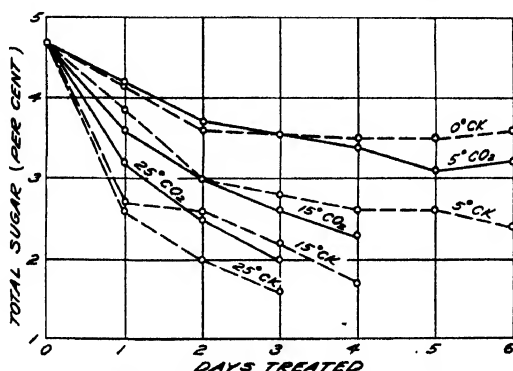


FIGURE 3.—Effect of exposure to 43 per cent CO_2 on total sugar in Early Evergreen sweet corn at various temperatures. (Solid lines, treated; broken lines, controls)

sour cherries received 45 per cent CO_2 for 2 and 4 days; the sweet cherries 44 per cent CO_2 for 2 and 3 days; and the peaches 35, 43, 44, and 47 per cent CO_2 for 1 and 2 days. The variation in

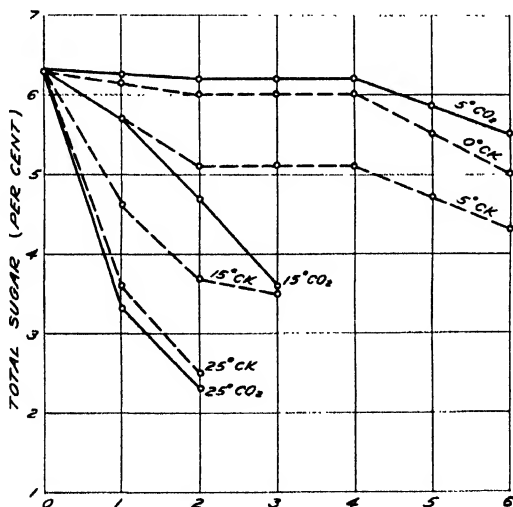


FIGURE 4.—Effect of exposure to 49 per cent CO_2 on total sugar in Golden Bantam sweet corn at various temperatures. (Solid lines, treated; broken lines, controls)

the results with these different percentages of CO_2 on the peaches was very slight, and they have been averaged for the reader's convenience.

TABLE 3.—*Reducing sugar, total sugar, and acid-hydrolyzable polysaccharides in cherries and peaches treated with CO₂*

[Expressed as percentage of fresh weight]

Fruit	Treatment		Reducing sugar		Total sugar		Acid-hydrolyzable polysaccharides	
	CO ₂	Temperature (° C.) and time	In treated product	In controls	In treated product	In controls	In treated product	In controls
	Per cent							
Sour cherries	45	20°, 2 days	7.5	6.9	-----	-----	0.62	1.17
		15°, 2 days	7.5	7.3	-----	-----	.53	.69
		10°, 2 days	6.5	7.2	-----	-----	.63	.60
		5°, 4 days	6.5	7.8	-----	-----	.54	.49
		2°, 4 days	7.2	7.2	-----	-----	.55	.59
Sweet cherries (Bing variety)	44	20°, 2 days	12.0	13.2	-----	-----	.68	.88
		15°, 2 days	13.6	12.2	-----	-----	.78	.82
		10°, 2 days	13.0	12.4	-----	-----	.82	.84
		5°, 3 days	11.1	12.2	-----	-----	.73	.75
		0°, 3 days	11.5	11.8	-----	-----	.65	.68
Peaches (Belle)	* 5-47	20°, 1 day	2.2	2.2	6.7	7.1	.84	.89
		15°, 1 day	2.2	2.3	6.3	6.3	1.06	.95
		10°, 2 days	2.0	2.4	5.8	7.3	.83	.98
		5°, 2 days	2.2	2.2	7.3	6.8	.85	1.01
		0°, 2 days	2.2	2.0	7.1	7.0	.80	.78

* Average of four experiments in which the percentage of gas was 35, 43, 44, and 47, respectively.

Apparently the CO₂ produced no effect upon the carbohydrate content of the peaches and cherries. The percentage of sugar and acid-hydrolyzable polysaccharides remained practically constant for both lots at all temperatures with the sour cherries, sweet cherries, and peaches.

DISCUSSION

The fact that there were no significant effects on carbohydrate transformation in the cherries and peaches as a result of the CO₂ treatment may help to answer a question frequently asked concerning the effect of CO₂ on fresh fruits. In a previous paper (7) reference was made to the deleterious effect of too great a concentration of CO₂ on the flavor of certain fruits such as peaches, strawberries, and raspberries. The question naturally arises as to whether the actual food value is also affected. From the evidence presented, the carbohydrate content of peaches and cherries appears to undergo no significant change from the CO₂ treatment given them. It should be recalled that the after-harvest behavior of peaches and cherries is very different from that of seeds like peas and corn. In other words, fruits are not converting their sugar to starch after harvesting, as is the case with peas and corn, and the amount of sugar consumed in respiration does not seem to be influenced by the CO₂ treatment. With the fresh fruits the CO₂ serves its purpose by keeping them firmer for a longer time than would be the case with storage in air.

By far the most pronounced effect of CO₂ on carbohydrate content was noted with peas and corn. It is common knowledge that these vegetables rapidly lose their palatability after being harvested, especially if held at summer temperatures, and this loss of flavor is largely the result of the conversion of sugar to polysaccharides.

The results of this work indicate that the conversion of sugar to starch in peas and corn can be considerably retarded by treatment

with CO₂ of the proper concentration. The greatest effect on peas was noticed at temperatures of 15°, 20°, and 25° C. At 5° and 0° the effect was not marked. Treated Sutton Perfection peas at 20° were 18 per cent higher, and Alaska peas at 25° were 76 per cent higher in total sugar than were the controls. The results for the Nott Excelsior and Gradus peas were similar though not so pronounced.

With all of the sweet corn except Golden Bantam the proper concentration of CO₂ retarded the sugar loss at all temperatures employed. In contrast to the results with peas, the effect of CO₂ on sweet corn was even more pronounced at the lower temperatures. In the experiments on Early Evergreen and Golden Bantam the carbohydrate changes could be easily followed by means of the consecutive sampling of the same lot.

At the time of sampling, the fruits and vegetables were tested for flavor. In all the peaches treated with CO₂ at 15° and 20° C. there was noticed a distinctly overripe flavor; those at 10° were generally questionable or on the border line, whereas at 5° and 0° no effect was noticed. Cherries were generally unaffected by the CO₂ treatment.

Data on the effect of CO₂ on the flavor of peas and corn were obtained by the continual exposure to the gas indicated in Figures 1-4. Forty-seven per cent CO₂ for 2 days did not affect the flavor of Nott Excelsior peas held at 5°, 15°, and 25° C. By the end of 3 days the 15° and 25° lots were objectionable in flavor, but those at 5° were still satisfactory. The Gradus peas at 25° endured 44 per cent CO₂ for 2 days and those at 15° for 3 days without any noticeable effect on flavor. The flavor of the 25° lot was objectionable at the end of 3 days and that of the 15° lot by 4 days of treatment. The 5° lot was satisfactory in regard to flavor by the end of 4 days.

The treatment of the Alaska and Sutton Perfection peas was not continued long enough to obtain data on the effect on flavor.

The Early Evergreen sweet corn reacted similarly to peas under the CO₂ treatment, but the Golden Bantam variety proved a little more sensitive. Both varieties endured the CO₂ treatment (43 to 49 per cent) at 5° C. for 5 days without impairment of flavor. The flavor of the Early Evergreen corn at 5° was questionable at the end of 6 days. The Golden Bantam corn could not be sampled after the fifth day because of insufficient amount of material offered by the normally small ears. The 25° lots of Early Evergreen corn withstood the CO₂ treatment for 2 days and the 15° lot for 3 days without loss of palatability. With the Golden Bantam the limit of treatment was 1 day at 25° and 2 days at 15°.

The treatment of the Stowell Evergreen sweet corn was not continued long enough to obtain data on effect on flavor.

SUMMARY

Peaches, cherries, sweet corn, and garden peas were treated with carbon dioxide gas at different temperatures for periods of one to six days and subsequently analyzed for sugars and acid-hydrolyzable polysaccharides.

The temperatures employed were 0°, 5°, 10°, 15°, 20°, and 25° C. Treatment at the higher temperatures was usually for one to three days, and that at the lower temperatures was usually from two to six days.

No significant difference in percentage of reducing sugar, total sugar, or acid-hydrolyzable polysaccharides was observed when sour cherries, sweet cherries, and Belle peaches were treated with CO₂ in concentrations of from 35 to 47 per cent.

Similar concentrations of CO₂ retarded the rate of sugar loss in peas and sweet corn.

Treatment of peaches with 35 to 47 per cent CO₂ at the higher temperatures (15° and 20° C.) produced a characteristic overripe flavor. In similar experiments the flavor of cherries was not affected by the CO₂ treatment. Exposure to 43 to 47 per cent CO₂ for two days did not affect the flavor of Gradus and Nott Excelsior peas or Early Evergreen sweet corn when held at 25° and 15°. One day at 25° and two days at 15° were the limit of tolerance to CO₂ for Golden Bantam sweet corn. At 5° the flavor of both varieties of peas was normal for three days and that of both varieties of sweet corn for four days. Increasing the time of exposure in any of the above cases was usually detrimental to flavor.

Within the limit of tolerance the treated sweet corn seemed sweeter than the controls when sampled.

LITERATURE CITED

- (1) APPLEMAN, C. O., and ARTHUR, J. M.
1919. CARBOHYDRATE METABOLISM IN GREEN SWEETCORN DURING STORAGE AT DIFFERENT TEMPERATURES. *Jour. Agr. Research* 17:137-152, illus.
- (2) ——— and CONRAD, C. M.
1926. PECTIC CONSTITUENTS OF PEACHES AND THEIR RELATION TO SOFTENING OF THE FRUIT. *Md. Agr. Expt. Sta. Bul.* 283, 8 p., illus.
- (3) ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS.
1930. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS . . . COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. Ed. 3, 593 p., illus. Washington, D. C.
- (4) BIGELOW, W. D., and GORE, H. C.
1905. STUDIES ON PEACHES. I. COMPILED ANALYSES OF PEACHES. II. CHANGES IN CHEMICAL COMPOSITION OF THE PEACH DURING GROWTH AND RIPENING. III. EFFECT OF STORAGE ON THE COMPOSITION OF PEACHES. *U. S. Dept. Agr., Bur. Chem. Bul.* 97, 32 p.
- (5) BOSWELL, V. R.
1929. FACTORS INFLUENCING YIELD AND QUALITY OF PEAS—BIOPHYSICAL AND BIOCHEMICAL STUDIES. *Md. Agr. Expt. Sta. Bul.* 306, p. 341-382.
- (6) BROOKS, C., and COOLEY, J. S.
1917. TEMPERATURE RELATIONS OF APPLE-ROT FUNGI. *Jour. Agr. Research* 8:139-164, illus.
- (7) ——— MILLEN, E. V., BRATLEY, C. O., COOLEY, J. S., MOOK, P. V., and JOHNSON, H. B.
1932. EFFECT OF SOLID AND GASEOUS CARBON DIOXIDE UPON TRANSIT DISEASES OF CERTAIN FRUITS AND VEGETABLES. *U. S. Dept. Agr. Tech. Bul.* 318, 60 p., illus.
- (8) HARTMAN, H., and BULLIS, D. E.
1929. INVESTIGATIONS RELATING TO THE HANDLING OF SWEET CHERRIES WITH SPECIAL REFERENCE TO CHEMICAL AND PHYSIOLOGICAL ACTIVITIES DURING RIPENING. *Oreg. Agr. Expt. Sta. Bul.* 247, 38 p., illus.
- (8a) JONES, H. A., and BISSEON, C. S.
1932. CHANGES IN THE COMPOSITION OF THE GARDEN PEA AFTER HARVEST. *Plant Physiol.* 7:273-283. illus.
- (8b) KERTESZ, Z. I.
1930. THE CHEMICAL CHANGES IN PEAS AFTER PICKING. *Plant Physiol.* 5:399-412. illus.

- (9) KIDD, F., and WEST, C.
1930. THE GAS STORAGE OF FRUIT. II. OPTIMUM TEMPERATURES AND ATMOSPHERES. *Jour. Pomol. and Hort. Sci.* 8:67-77, illus.
- (10) STEVENS, N. E., and HIGGINS, C. H.
1919. TEMPERATURE IN RELATION TO QUALITY OF SWEETCORN. *Jour. Agr. Research* 17:275-284, illus.
- (11) STRAUGHN, M. N.
1907. SWEETCORN INVESTIGATIONS. *Md. Agr. Expt. Sta. Bul.* 120, p. [37]-78, illus.
- (12) ——— and CHURCH, C. G.
1909. THE INFLUENCE OF ENVIRONMENT ON THE COMPOSITION OF SWEET-CORN, 1905-1908. *U. S. Dept. Agr., Bur. Chem. Bul.* 127, 69 p., illus.
- (13) THORNTON, N. C.
1930. CARBON-DIOXIDE STORAGE OF FRUITS, VEGETABLES, AND FLOWERS. *Indus. and Engin. Chem.* 22:1186-1189, illus.

RHIZOCTONIA BOTTOM ROT AND HEAD ROT OF CABBAGE¹

By F. L. WELLMAN²

Associate Pathologist, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The damping-off or wire-stem disease of cabbage (*Brassica oleracea* L.) seedlings, caused by the Rhizoctonia stage of *Corticium vagum* Berk. and Curt., has been known for a long time and has been studied critically by Gratz (4).³ For several years the writer has observed and investigated two other phases of Rhizoctonia injury to cabbage, known as bottom rot and head rot. The disease called head rot was first described by the writer (13) in 1927. In 1931 Weber (14) reported in abstract a Rhizoctonia bottom rot in Florida.

The present paper reports the results of studies of the cabbage-growing sections of Wisconsin, Indiana, and Illinois and parts of Iowa and Ohio. Each year from 1923 to 1928, inclusive, damping off, or wire stem, and bottom rot have been observed. Head rot, however, has been irregular in occurrence. It was seen first in 1923 and again in 1924. In 1925 it caused losses of 5 to 9 per cent in many fields in northeastern Illinois and southeastern Wisconsin. In 1926 it was less abundant, and in 1927 and 1928 fields were practically free from it.

DESCRIPTION OF RHIZOCTONIA ROTS OF CABBAGE

BOTTOM ROT

Usually there is little trouble from Rhizoctonia until the transplanted seedlings are large enough to begin to shade the ground. The disease first appears on the basal portions of the leaves next to the soil, the ventral side of the midrib often being the first part of the leaf attacked. The resulting lesions are sunken, black, and sharply elliptical, with their long axes parallel to the sides of the midrib. Round black spots appear on the basal parts of the leaf lamina, and these areas gradually enlarge. The diseased spots usually have over them sparse weblike surface mycelia. Eventually there is a general decay of the base of the leaf and the tissues become black and easily torn. The distal part of the leaf finally turns yellow, and the whole leaf droops, may dry up and drop off, and is often covered over by soil during subsequent cultivation. The *Alternaria* leaf spot (*Alternaria brassicae* (Berk.) Sacc.), which has been described by Weimer (15), may be confused with Rhizoctonia. The slowly growing

¹ Received for publication Mar. 30, 1932; issued October, 1932. Cooperative investigations between the Wisconsin Agricultural Experiment Station and the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Published with the approval of the director of the Wisconsin Agricultural Experiment Station.

² The writer acknowledges his indebtedness to Dr. J. C. Walker, of the University of Wisconsin, for criticism and aid in the preparation of the manuscript. He is indebted also to the plant pathology department of the University of Wisconsin and to J. Monteith, Jr., formerly of the Division of Forage Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, for cultures of *Rhizoctonia solani* supplied by them for comparison with the writer's cultures.

³ Reference is made by number (italic) to Literature Cited, p. 469.

lesion produced by the *Alternaria* becomes light colored and papery and is less extensive than the *Rhizoctonia* lesion. *Rhizoctonia* infects the bases of the leaves next to the ground first, whereas *Alternaria* attacks the upper surface of the tip and other distal points with equal facility. *Rhizoctonia* bottom rot is not necessarily followed by head rot.

HEAD ROT

Head rot (fig. 1) occurs spasmodically in the field under conditions as yet not well defined. If bottom rot occurs on a headed plant in the field and proper conditions prevail, such as high relative humidity and warm weather, hyphae of the organism grow along the stem without producing decay and infect other leaves farther up. These weaken the midribs and the leaves droop. If feces of cabbage worms lodge on the dorsal side of the petioles and on leaf blades, growth of the organism is stimulated and its head-rotting action is materially hastened. As the bases of the first cover leaves of the head are attacked, the exposed tips on top of the head turn yellow and tend to dry up. A few hyphae spread rapidly between the leaves of the head, and lesions are produced. The latter are mere punctations (fig. 2) at first, but later expand and coalesce and have scant strands of hyphae over them. The lesions are dark and sunken and have a suggestion of concentric zonation about a raised center at the point of infection. Hyphae may later grow out from such spots and produce numerous new lesions near the old. These diseased spots finally become confluent. If the weathered leaves on a diseased head are pulled apart a not unpleasant odor like that of boiled cabbage is noticed. As decay progresses, a weblike dark-colored mycelium develops between the diseased leaves. As the head rot advances only the leaf tissues are affected, while the stem and core of the head are not diseased. The head remains, therefore, conspicuous, upright, dark colored, and studded with small brown sclerotia.

The *Corticium* stage of *Rhizoctonia solani* Kühn is found early in the cabbage fields of Wisconsin and becomes more prevalent as the season advances. It occurs on the ventral sides of the lower leaves as a superficial gray or chalk-colored membranous growth, which can be easily stripped from its support. It is also found growing over the surface of the soil in protected places and attached to cabbage stems at the ground line.

COMPARISON WITH OTHER HEAD ROTS

The *Rhizoctonia* head rot differs from other head rots of cabbage in a number of details. The foliage leaves about an attacked head droop in a characteristic manner but may not drop completely from the plant. (Fig. 1, A.) The decaying tissues are more pliable and tough than those produced in watery soft rot or drop (*Sclerotinia sclerotiorum* (Lib.) Mass.) and in bacterial soft rot (*Bacillus carotovorus* L. R. Jones). In Wisconsin two head rots, one caused by *Sclerotinia* and one caused by *Rhizoctonia*, produce sclerotia. *Rhizoctonia* sclerotia are the smaller, the more irregular, and the more numerous. *Rhizoctonia* decay is neither slimy, as in bacterial soft rot, nor watery and translucent, as in watery soft rot and in head rot caused by species of *Pythium* (2). The infected tissues turn dark and shrink as they decay. In bacterial soft rot the odor of decay is

very noticeable, but in neither of the other head rots mentioned does the odor offend greatly. In *Sclerotinia* and *Pythium* rots the aerial mycelium is white and is most abundant in the former. (Fig. 1, B.)



FIGURE 1.—Comparison of *Rhizoctonia* and *Sclerotinia* head rots of cabbage in the field. A.—*Rhizoctonia* head rot: Diseased head erect, somewhat shrunk, roughened, and dark colored; foliage leaves droop but remain attached. B.—*Sclerotinia* head rot: Some foliage leaves broken off to allow for photographing head; profuse white aerial mycelium; only a few large black sclerotia; leaves not weakened at base as in *Rhizoctonia* head rot.

Rhizoctonia upon infecting a mature cabbage head attacks only the leaf tissues, whereas bacterial black rot (*Bacterium campestris* (Pam.) E. F. Smith) is characterized by blackened vascular bundles. In cot-

tony rot, watery soft rot, and bacterial soft rot there may be decay of the stem.

THE CAUSAL ORGANISM

ISOLATION

Numerous isolations were made from cabbage seedlings affected with damping off and with wire stem, from leaves affected with bottom rot, and from heads affected with head rot. The *Corticium* stage of apparently the same organism was commonly found in the autumn on leaves showing signs of bottom rot and around the base of the plants. Four cultures were made from single spores and several



FIGURE 2.—Lesions on surface of inside leaf of cabbage head recently infected with *Rhizoctonia solani*. Older lesions dark and sunken; some show rough concentric zonation around a raised center. Small punctations about large spots are new infections from hyphae advancing beyond older lesions. Such groups of lesions expand rapidly, coalesce, and may involve the whole head. Sclerotia nearly always form at the point of infection.

from bits of the mycelium of the hymenial layer. All these cultures when compared with cultures known to be those of *Rhizoctonia solani* and with descriptions by Kühn (5), Duggar (3), Matsumoto (7), and Burt (1), were found to belong to this species.

PATHOGENICITY

Numerous cultures from the sources listed above were used in inoculation experiments upon cabbage plants at various stages of growth, including 15 to 20 flats of young seedlings, a large number of partly grown potted plants, and over 200 cabbage heads, some growing in the field and some cut and placed under bell jars in the laboratory. The results of each experiment were checked with control

plants. The inoculations were generally successful. All strains produced typical damping off of the seedlings, bottom rot of older plants, and typical head rot of heads approaching maturity or already harvested. The results showed conclusively that bottom rot and head rot are caused by the strains of the imperfect stage of the fungus *Corticium vagum* isolated from cabbage. They show furthermore that the causal organism is identical with that causing the *Rhizoctonia* damping off or wire stem of cabbage seedlings.

PHYSIOLOGIC SPECIALIZATION OF RHIZOCTONIA STRAINS

Physiologic specialization in fungi, in which certain morphologic species are subdivided into forms exhibiting distinct parasitic specialization, is a well-known phenomenon. It has been studied in *Rhizoctonia solani* by several workers, the most important contributions being those of Duggar (3), Matsumoto (7), and Peltier (10). The physiologic reactions of strains of the fungus in pure culture as a measure of biologic specialization has not yet received adequate attention; the pure-culture work of Matsumoto (7) and of Monteith and Dahl (9) are thus far the most important contributions to this problem.

Physiologic specialization of *Rhizoctonia solani* on crucifers has received only incidental attention. Peltier (10) found that a strain isolated from cauliflower, though able to infect seedlings of a fairly wide range of host plants, appeared to attack the Cruciferae most severely. Gratz (4), studying strains isolated from a number of host plants, found that the *Rhizoctonia* causing wire stem of cabbage was probably pathogenic on that plant only, and that the typical potato *Rhizoctonia* did not cause wire stem of cabbage. Van der Meer (8) found that strains of *Rhizoctonia* obtained from potato and cauliflower "were physiologically distinct from each other." Lauritzen's (6) study of *Rhizoctonia* rot of turnips showed that the potato and turnip strains were pathogenic only on the hosts from which they were isolated. The question of physiologic specialization inevitably presented itself in connection with the present study of *Rhizoctonia* causing bottom rot and head rot of cabbage.

Sixty-nine isolations of *Rhizoctonia* from a number of hosts were studied. In this discussion the data from only 15, representing isolations from 6 different hosts, will be considered. The results of comparative inoculations at various stages in the development of cabbage, potato, and lettuce are summarized in Table 1. It is to be noted that the cabbage isolations were practically nonpathogenic to potato and that the potato isolations were nonpathogenic to cabbage. This is in accord with the results of Gratz (4) and of Lauritzen (6) noted above, indicating that isolations of *Rhizoctonia* from cruciferous hosts were rather specific to those plants. Isolations from bean, beet, pea, and lettuce likewise failed to cause head rot or bottom rot of cabbage, although that from lettuce did cause damping off of small cabbage seedlings. On the other hand, all the cabbage strains were fairly pathogenic to lettuce. These results, though limited, indicate that only certain strains of *Rhizoctonia solani* are capable of causing head rot and bottom rot of cabbage.

TABLE 1.—*Specialized pathogenicity of certain representative strains of Corticium vagum*

Strain		Results of inoculations ^a										
No.	Host from which obtained	Disease on host	On cabbage			On potato				On lettuce		
			Head rot	Bottom rot	Seedling damping off	Under-ground shoot decay	Aerial stem lesion	Aerial leaf rot	Sclerotia on tubers	Head rot	Foliage leaf rot	Seedling damping off
1	Bean.....	—	—	—	—	—	—	—	—	—	—
2	do.....	—	—	—	—	—	—	—	—	—	—
3	Beet.....	—	—	—	—	—	—	—	—	—	—
4	Pea.....	—	—	—	—	—	—	—	—	—	—
5	Lettuce.....	—	—	—	—	—	—	—	—	—	—
6	do.....	—	—	—	—	—	—	—	—	—	—
7	Potato.....	—	—	—	—	—	—	—	—	—	—
8	Sclerotia on tuber.....	—	—	—	—	—	—	—	—	—	—
9	do.....	—	—	—	—	—	—	—	—	—	—
10	do.....	—	—	—	—	—	—	—	—	—	—
11	Cabbage.....	—	—	—	—	—	—	—	—	—	—
12	do.....	—	—	—	—	—	—	—	—	—	—
13	do.....	—	—	—	—	—	—	—	—	—	—
14	do.....	—	—	—	—	—	—	—	—	—	—
15	do.....	—	—	—	—	—	—	—	—	—	—

^a + indicates infection; — indicates no infection.^b Sclerotia small and few in number.

ENVIRONMENT IN RELATION TO DISEASE PRODUCTION BY THE
CABBAGE RHIZOCTONIA

MOISTURE RELATIONSHIPS

In his studies of damping off Gratz (4) found that *Rhizoctonia* required a high moisture content of the soil. Either drought or excess of water in the soil hindered the development of the disease. The writer's observations corroborate these findings.

To determine the effect of relatively high and low moisture content of the air on the *Rhizoctonia* producing bottom rot and head rot, a number of experiments were performed. Heads of cabbage obtained on the Madison, Wis., market were taken as host material, and two strains of the fungus were used. After the outer leaves had been carefully removed under aseptic conditions, inoculations at seven points on the top of each head were made from *Rhizoctonia* cultures. The inoculum consisted of surface sclerotia and bits (about 1 cm square) of the culture medium covered with young growing hyphae. Half of the inoculated heads were placed under bell jars and half were left uncovered in the comparatively dry laboratory air. The temperature of the room was about 21° C. Under the bell jars fungal hyphae could be noted within 24 hours, extending out from sclerotia and bits of agar culture, and in three days the leaf tissue showed symptoms of attack and the organism developed rapidly. Release of water from the host tissue appeared much accelerated with increased decay, water drops gathering in much greater quantities on the inside of bell jars over decaying heads than over uninoculated individuals. On the inoculated heads left uncovered the inoculum dried up and no *Rhizoctonia* decay resulted.

Plants growing in sterilized soil in the greenhouse were likewise used in determining the relation of moist atmosphere to disease production. These plants, of an early variety of cabbage, had grown rapidly and were forming small heads. Greenhouse temperatures ranged between 18° and 24° C. Inoculations were made with young sclerotia from pure cultures of a cabbage head-rot strain of *Rhizoctonia*. Inoculum was placed in the forming head and on foliage leaves. Controls were inoculated with bits of sterile agar. The soil was kept wet and some of the plants were covered with a glass-sided box, and others were left uncovered. The *Rhizoctonia* organism produced typical head rot and bottom rot on all the inoculated plants kept under the moist chamber; a few unprotected plants inoculated in the forming head showed signs of becoming diseased, though the symptoms were not advanced. It appears that a very humid atmosphere is most conducive to the progress of both diseases and is particularly important for the development of head rot.

TEMPERATURE RELATIONSHIPS

Richards (11), in 1921, reported studies on the relation of soil temperature to injury of potato stems by *Corticium vagum* (*Rhizoctonia solani*). He found that the organism caused lesions over a wide range of temperatures (9° to 27° C.), that the optimum temperature for pathogenicity was 18°, and that above 24° *Rhizoctonia* was not a serious factor in disease production. In 1923, he (12) again reported studies of *Rhizoctonia*, this time on pea and bean. As before, he found that temperature had a very definite effect on disease produc-

tion. No infection occurred below 9° or above 29°. The optimum may be placed at 18°. In pure culture the range differed, the minimum temperature for growth being 4.6°, the optimum from 25° to 27°, and the maximum 32.6°. Gratz (4), in 1925, found that *Rhizoctonia* damping off of cabbage had quite definite temperature relationships. The minimum for disease production was below 10°, the optimum from 15° to 30°, and the maximum from 31° to 32°. In pure culture the organism grew at a minimum of approximately 9°, an optimum between 22° and 26°, and a maximum of slightly above 31°. In 1928, Monteith and Dahl (9) reported temperature relationships of pure cultures of nine strains of *Rhizoctonia solani* from grass, pea, and potato. In general these strains grew at a minimum temperature of less than 10°, an optimum of 25° to 30°, and a maximum of 35°.

Temperature relationships determined by the writer for the organism causing bottom rot and head rot of cabbage are practically the same as those found by Gratz (4). In pure culture on potato-dextrose agar the cabbage *Rhizoctonia* produced growth at 8° C. At 9° the fungus grew very slowly. Between 12° and 22° rapidity of growth increased enormously. The most rapid growth was above 23° and below 27°. At 29° the rate of growth was materially lessened, at 30° it dropped still further, and at 33° no growth occurred. General field observations indicated that *Rhizoctonia* was most active during warm weather. Headed plants were potted, inoculated with sclerotia from pure cultures of *Rhizoctonia* causing head rot, and placed in greenhouses at temperatures ranging from 10° to 33°. Inoculated heads decayed slowly at a temperature of 12°. At 15° to 20° the disease developed at a slightly more rapid rate. At 21° to 23° disease production was further enhanced. The optimum temperature for head rot was determined to be from 23° to 27°. Above 30° decay was considerably lessened, and at 32° there was none.

In a further study of temperature relations, bleached leaves aseptically removed from the inside of heads of market cabbage were put in sterilized moist chambers and inoculated with pure cultures of two strains of *Rhizoctonia* obtained from cabbage plants affected with head rot. The moist chambers were then held at constant temperatures. Temperature relationships for decay were the same as previously determined, except that the minimum temperature was placed at about 9° C. and the optimum temperature narrowed down to between 25° and 27°.

SUMMARY

The present paper describes two diseases of cabbage, *Rhizoctonia* bottom rot and head rot, and presents the results of recent studies on both of these diseases, which are caused by *Rhizoctonia solani* Kühn, the imperfect form of *Corticium vagum* Berk. and Curt. Bottom rot attacks cabbage every year, but head rot is spasmodic in its occurrence. It was proved by isolation and inoculation experiments that these two diseases of older plants in the field are caused by the same strain of *Rhizoctonia* that produces damping off, or wire stem, of cabbage seedlings.

A number of strains of *Rhizoctonia solani* were obtained and studied for evidence of physiologic specialization. It appears that strains of the organism are most pathogenic upon the hosts from which they were originally isolated.

Environmental factors were studied in relation to disease production. It was found that a relatively large amount of moisture is necessary for optimum disease production by the fungus. *Rhizoctonia* bottom rot and head rot were produced at temperatures ranging from 9° C. to somewhat less than 32°. The optimum temperature for disease development is between 25° and 27°.

LITERATURE CITED

- (1) BURT, E. A.
1918. CORTICIUMS CAUSING PELLICULARIA DISEASE OF THE COFFEE PLANT, HYPOCHNOSE OF POMACEOUS FRUITS, AND RHIZOCTONIA DISEASE. *Ann. Missouri Bot. Gard.* 5:119-132, illus.
- (2) DRECHSLER, C.
1925. WIRE STEM OF CABBAGE HEADS. *Phytopathology* 15:[482]-485, illus.
- (3) DUGGAR, B. M.
1915. RHIZOCTONIA CROCORUM (PERS.) DC. AND R. SOLANI KÜHN (CORTICIUM VAGUM B. AND C.) WITH NOTES ON OTHER SPECIES. *Ann. Missouri Bot. Gard.* 2:403-458, illus.
- (4) GRATZ, L. O.
1925. WIRE STEM OF CABBAGE. *N. Y. Cornell Agr. Expt. Sta. Mem.* 85, 60 p., illus.
- (5) KÜHN, J.
1858. DIE KRANKHEITEN DER KULTURGEWÄCHSE, IHRE URSACHEN UND IHRE VERHÜTUNG. 312 p. Berlin.
- (6) LAURITZEN, J. I.
1929. RHIZOCTONIA ROT OF TURNIPS IN STORAGE. *Jour. Agr. Research* 38:93-108, illus.
- (7) MATSUMOTO, T.
1921. STUDIES IN THE PHYSIOLOGY OF THE FUNGI. XII. PHYSIOLOGICAL SPECIALIZATION IN RHIZOCTONIA SOLANI KÜHN. *Ann. Missouri Bot. Gard.* 8:1-62, illus.
- (8) MEER, J. H. H., VAN DER.
1926. RHIZOCTONIA—EN OLPIDIUM—AANTASTING VAN BLOEMKOOLPLANTEN. *Tijdschr. Plantenziekten* 32:[209]-242, illus.
- (9) MONTEITH, J., JR., and DAHL, A. S.
1928. A COMPARISON OF SOME STRAINS OF RHIZOCTONIA SOLANI IN CULTURE. *Jour. Agr. Research* 36:897-903, illus.
- (10) PELTIER, G. L.
1916. PARASITIC RHIZOCTONIAS IN AMERICA. *Ill. Agr. Expt. Sta. Bul.* 189, p. 283-390, illus.
- (11) RICHARDS, B. I.
1921. PATHOGENICITY OF CORTICIUM VAGUM ON THE POTATO AS AFFECTED BY SOIL TEMPERATURE. *Jour. Agr. Research* 21:459-482, illus.
- (12) ———
1923. SOIL TEMPERATURE AS A FACTOR AFFECTING THE PATHOGENICITY OF CORTICIUM VAGUM ON THE PEA AND BEAN. *Jour. Agr. Research* 25:431-450, illus.
- (13) WALKER, J. C.
1927. DISEASES OF CABBAGE AND RELATED PLANTS. *U. S. Dept. Agr. Farmers' Bul.* 1439, 30 p., illus. (Section on *Rhizoctonia* Head Rot prepared by F. L. Wellman, p. 29.)
- (14) WEBER, G. F.
1931. BOTTOM ROT OF CABBAGE CAUSED BY CORTICIUM VAGUM. (Abstract) *Phytopathology* 21:117.
- (15) WEIMER, J. L.
1924. ALTERNARIA LEAFSPOT AND BROWNROT OF CAULIFLOWER. *Jour. Agr. Research* 29:421-441, illus.

THE DIGESTIVE ENZYMES OF THE COLORADO POTATO BEETLE AND THE INFLUENCE OF ARSENICALS ON THEIR ACTIVITY¹

By DAVID E. FINK²

*Entomologist, Division of Insect Toxicology and Physiology, Bureau of Entomology,
United States Department of Agriculture*

INTRODUCTION

In a previous investigation the writer (6)³ found that, in general, arsenic caused an inhibition of the respiratory metabolism of insects. This led to the assumption that arsenic may exert its toxic action through its chemical affinity for some substance in the protoplasm that normally is of vital importance to respiration. Glutathione, known to be of importance in cell oxidation and reduction and detected by the writer (7) in insect tissue, was found to be reduced in quantity when arsenicals were fed or injected into insects (8). Furthermore, Voegtlin, Dyer, and Leonard (18, p. 304) have stated that

arsenic in the form of the trivalent directly toxic oxide modification exerts its action on mammalian protoplasm through its chemical affinity to the SH group of reduced glutathione, thus interfering with the normal equilibrium between oxidized and reduced glutathione, as a result of which the tissue dies of asphyxia.

The foregoing studies indicate that the reaction between arsenic and reduced glutathione may be an important part of the mechanism of the toxic action of arsenic. Parfentjev and Devrient (10), however, believe that the effect of arsenic on the respiratory metabolism of insects can not explain the mechanism of its toxic action. They suggest that the loci of its action should be sought elsewhere. Since our knowledge of the mechanism of the toxic action of arsenic is contradictory and incomplete, it seems desirable to exhaust every possible line of investigation before assigning to any one reaction a predominant rôle in the total effect of arsenic.

If arsenic interferes with the activity of respiratory catalysts, perhaps it also affects certain digestive enzymes. A study of the literature failed to reveal a single experiment on the effect of arsenic on the digestive enzymes of insects. Accordingly, the writer has made a study of the effect of arsenicals on the digestive enzymes of the Colorado potato beetle, *Leptinotarsa decemlineata* (Say). In this investigation it was necessary first to determine the presence and activity of the digestive enzymes in the normal insect and afterwards to investigate the influence of arsenicals upon their activity.

Received for publication Feb. 15, 1932; issued October, 1932.

¹ The writer expresses his gratitude to J. E. Graf, formerly in charge of the Division of Truck Crop Insects, for the opportunity and encouragement to continue these physiological studies. During the summers of 1928 and 1929 the writer was assisted in the experimental work by H. Beerman, C. E. Herber, and H. Rosen.

² Reference is made by number (italic) to Literature Cited, p. 481.

Journal of Agricultural Research,
Washington, D. C.

Vol. 45, No. 8
Oct. 15, 1932
Key No. K-231

THE DIGESTIVE ENZYMES OF THE NORMAL COLORADO POTATO BEETLE**METHODS OF INVESTIGATION**

Active, feeding potato beetles were deprived of food for several days and were given large quantities of water during the same period to clear the digestive tract of food material. Repeated dissections of beetles have shown that a period of two to three days is usually sufficient for the removal of the greater part of the food. The number of beetles dissected at any one time depended upon the extensiveness of the experiment, and ranged from about 100 to 300.

The entire digestive tract was removed and divided into three parts, the fore-gut, mid-gut, and hind-gut. The fore-gut is about 4 mm in length, the mid-gut about 16 mm, and the hind-gut about 8 mm. Dissections were made in a 0.2 per cent sodium chloride solution. Any food and excrement remaining were removed, and the dissected parts rinsed in several changes of the salt solution and finally in distilled water. The three parts of the digestive tract thus prepared were divided into two equal samples. The three parts of the first sample were ground separately with clean washed sand in a mortar. Each part was placed in an Erlenmeyer flask, and the contents of each of the three flasks were diluted with a 50 per cent glycerol solution in the proportion of 1 c c of the solution to 1 mid-gut, 4 fore-guts, and 2 hind-guts. The other sample was similarly treated, except that distilled water was used as the diluent. The tissue suspensions prepared in this manner were fairly homogeneous. A layer of toluene (5 c c) was added to the contents of each of the six flasks as a preservative. The glycerol-tissue suspension was subsequently used in the experimental work on proteolytic enzymes, and the distilled water-tissue suspension was used in the work on enzymes that split carbohydrates. This division was necessary because glycerol solutions in certain concentrations are known to inhibit the activity of certain enzymes.

A suspension was also prepared of the contents of the digestive tract that usually ooze from the mouths of active potato beetles when handled. This regurgitated liquid was collected by means of a fine-tipped capillary pipette held close to the mouths of active beetles. It was then diluted with distilled water to a yellow suspension which contained very active enzymes.

To determine the effect of age of the tissue suspensions on the activity of the enzymes, some experiments were made with a tissue suspension from 1 day to 3 weeks old.

To determine the presence or absence of enzymes, preliminary experiments by the general micro and macro methods described by Swingle (17) were tried with these tissue suspensions. Afterwards other methods and modifications were adopted for estimating the extent of digestion caused by a given enzyme. These methods will be described in detail under each enzyme discussed.

A striking characteristic of enzyme activity is the fact that it is limited to a definite range of hydrogen-ion concentration. One of the aims of the writer was to determine the hydrogen-ion concentration of optimum activity and the effective pH range for each enzyme studied in this investigation. In preliminary experiments the writer was guided in the choice of pH range by the large amount of published

information on the effective pH ranges for different enzymes. Clark's (4, p. 104) phthalate and phosphate mixtures were employed as buffers. In some experiments the solutions were not buffered but were adjusted to a desired hydrogen-ion concentration by the addition of hydrochloric acid or sodium carbonate. The hydrogen-ion concentrations of the solutions before and during their use in the experiments were frequently checked against those of standard buffer solutions, generally by the usual spot plate and potentiometer methods (9).

EXPERIMENTAL WORK

HYDROGEN-ION CONCENTRATION OF TISSUE SUSPENSION

The tissue suspensions in distilled water described in the preceding section were used first for the determination of their hydrogen-ion concentration. Samples were tested by the potentiometer method. The pH of the distilled water was adjusted to 7.0 by means of dilute NaOH. The average pH of the tissue suspension of the fore-gut was 6.42; of the mid-gut, 5.00; and of the hind-gut, 5.40. The average pH of the suspension of regurgitated liquid was 5.00.

TABLE 1.—*Digestion of starch by amylase obtained from a mid-gut tissue suspension prepared from normal and arsenic-fed potato beetles*

Experiment No.	Treatment of beetles	Period of digestion	Extent of digestion * at pH of -				
			6.0	6.6	7.0	7.6	8.0
2	{ Normal	<i>Minutes</i>					
		5	0		20	20	20
		25	20		40	60	35
		50	30		50	70	40
		70	40		60	80	45
	115	60		80	100	60	
{ Control		0	0	0	0	0	
3	{ Normal ^b	5	40	20	20	20	
		15	60	40	40	40	
		25	100	65	50	45	
		60		100	80	55	
		115			100	80	
	{ Control		0	0	0	0	
5	{ Fed Paris green.....	5	0	0	0	10	20
		25	0	20	20	20	30
		50	20	30	35	40	40
		70	40	40	60	60	55
		115	60	65	70	75	60
	{ Control		0	0	0	0	0
6	{ Fed lead arsenate	5	10	20	20	20	20
		25	20	30	40	40	30
		50	30	40	50	60	35
		70	40	50	70	75	50
		115	60	70	80	85	70
	{ Control		0	0	0	0	0

* Complete digestion equals 100.

^b Regurgitated liquid was used instead of tissue suspension.

AMYLASE

Two cubic centimeters of tissue suspension, 2 c c of buffer solution of a desired hydrogen-ion concentration, and 0.5 c c of a 0.5 per cent soluble-starch solution were accurately measured into each of a set of test tubes. A similar set containing boiled tissue suspension was used as a control. These solutions were covered with a layer of toluene and placed in a water bath at 37° C. A drop of an iodine solution (1 per cent iodine-potassium iodide solution) was added

to each test tube. At certain intervals, shown in Table 1, the progress of the hydrolysis of starch by this enzyme was followed by comparing the colors of the solutions with those of a set of five standards ranging from a deep blue to light yellow. The standards were prepared by adding bromothymol blue to buffered solutions of suitable hydrogen-ion concentration. As a result of preliminary experiments, numbers were assigned to the standards as follows: Deep blue, 0; light blue, 20; purple, 40; yellow, 60; light yellow, 80; colorless, 100. The interpolated numbers given in Table 1 indicate estimates of extent of digestion in a digest tube when at the given interval of time the color of the solution in the tube did not match that of a standard. The hydrogen-ion concentration of the solution reaching the achromic point in the shortest interval of time approximated the hydrogen-ion concentration of optimum activity of the enzyme. Before the progress of digestion in a tube was recorded, several more drops of the iodine solution were added to make certain that the color change was due to digestion of starch and not to absorption of iodine.

ENZYMES HYDROLYZING DISACCHARIDES

During the hydrolysis of disaccharides by different enzymes, glucose is produced. For example, lactase hydrolyzes lactose into galactose and glucose, maltase hydrolyzes maltose into two molecules of glucose, and invertase hydrolyzes sucrose into fructose and glucose. It was found convenient to take advantage of this fact for the purpose of detecting and estimating the relative activity of the different enzymes hydrolyzing disaccharides. Benedict's quantitative solution (a modification of Fehling's solution) was used. One cubic centimeter of this reagent, when reduced, is equivalent to 2 mg of glucose. After determining the number of cubic centimeters of an incubated mixture required to reduce 2 c c of Benedict's reagent, one is able to express the extent of digestion in milligrams of glucose per cubic centimeter of incubated mixture.

The writer found the following method convenient: Into a series of test tubes 2 c c of Benedict's solution was accurately measured by means of a burette. In addition, each tube received 0.5 g of anhydrous sodium carbonate and a little powdered pumice, and the contents were heated to boiling over a free flame until the carbonate was completely dissolved. By means of a 1, 2, or 5 c c Mohr pipette filled to the zero mark, the incubated mixture to be tested was run into the boiling reagent (the test tube being held just above the tip of a free flame), rather rapidly at first, then more slowly as the color began to fade, and finally drop by drop until a white precipitate formed and the solution became colorless. A pipette graduated to 0.01 c c was used to obtain the end point. The error in determining the end point was not greater than 0.03 c c of tissue suspension. The reduction of 2 c c of Benedict's reagent was accomplished under these conditions by exactly 4 mg of glucose.

The figures in milligrams of glucose are an indication of the relative hydrolyzing activity of the enzyme in this incubated mixture at the hydrogen-ion concentration of the experiment and are not to be construed as indicating actual quantities of enzyme per cubic centimeter of tissue suspension.

LACTASE

Into a series of test tubes, 2 c c of tissue suspension, 2 c c of buffer solution of a desired hydrogen-ion concentration, and 5 c c of a 1 per cent solution of lactose were accurately measured, covered with a layer of toluene, and placed in a water bath at 37° C. Controls received the same quantity of boiled tissue suspension. After a certain period measured portions of the digest were removed and tested with Benedict's reagent to determine the extent of digestion. Table 2 shows that lactase was present in the mid-gut and active from pH 4.0 to 8.0, the optimum activity occurring from pH 6.0 to 8.0.

MALTASE

The procedure for determining the presence and activity of maltase was the same as that described under lactase, except that a 1 per cent maltose solution was used as substrate. Table 3 shows that maltase was present in the mid-gut. Its active range was found to be from pH 6.6 to 8.0, the optimum activity being at pH 7.6.

INVERTASE

The procedure for determining the presence and activity of invertase was as described under lactase, a 1 per cent solution of sucrose being used as substrate. As shown in Table 4, this enzyme was present in the mid-gut and was active throughout the pH range used (pH 4.0 to 8.0), being most active between pH 5.0 and 7.0, with optimum activity at pH 6.0.

TABLE 2.—*Hydrolysis of lactose by lactase obtained from a mid-gut tissue suspension prepared from normal and arsenic-fed potato beetles*

Experiment No.	Treatment of beetles	Extent of hydrolysis * at pH of—				
		4.0	5.0	6.0	7.0	8.0
		Mg	Mg	Mg	Mg	Mg
11	Normal.....	3.41	3.62	4.06	4.00	4.08
	Control.....	3.00	3.05	3.08	3.02	3.06
12	Normal.....	2.53	2.84	2.90	2.90	2.91
	Control.....	2.35	2.40	2.45	2.23	2.30
15	Fed Paris green.....	3.10	3.25	3.30	3.55	3.08
	Control.....	3.08	3.10	3.02	3.10	3.12
16	Fed Paris green.....	3.40	3.45	3.48	3.54	3.59
	Control.....	3.21	3.18	3.25	3.22	3.21
17	Fed lead arsenate.....	2.78	2.80	2.90	2.98	2.98
	Control.....	2.69	2.75	2.71	2.70	2.69
18	Fed lead arsenate.....	2.89	2.95	2.98	3.08	3.14
	Control.....	2.72	2.76	2.79	2.67	2.71

* In terms of milligrams of glucose per cubic centimeter of incubated mixture.

TABLE 3.—*Hydrolysis of maltose by maltase obtained from a mid-gut tissue suspension prepared from normal and arsenic-fed potato beetles*

Experiment No.	Treatment of beetles	Extent of hydrolysis * at pH of -						
		4.0	5.0	6.0	6.6	7.0	7.6	8.0
		Mg	Mg	Mg	Mg	Mg	Mg	Mg
23	Normal.....	2.30	2.30	2.30	2.50	2.55	2.70	2.50
	Control.....	2.20	2.18	2.20	2.19	2.21	2.16	2.20
24	Normal.....	2.20	2.23	2.28	2.46	2.50	2.80	2.68
	Control.....	2.12	2.18	2.14	2.10	2.11	2.13	2.10
27	Fed Paris green.....	3.17	3.20	3.33	3.38	3.48	3.59	3.36
	Control.....	3.12	3.15	3.09	3.14	3.18	3.17	3.13
28	Fed Paris green.....	3.39	3.27	3.41	3.39	3.56	3.67	3.40
	Control.....	3.20	3.25	3.24	3.20	3.27	3.29	3.19
29	Fed lead arsenate.....	2.81	2.89	2.88	2.97	2.94	2.90	2.98
	Control.....	2.79	2.76	2.80	2.69	2.82	2.78	2.80

* In terms of milligrams of glucose per cubic centimeter of incubated mixture.

TABLE 4.—*Hydrolysis of sucrose by invertase obtained from a mid-gut tissue suspension prepared from normal and arsenic-fed potato beetles*

Experiment No.	Treatment of beetles	Extent of hydrolysis * at pH of—				
		4.0	5.0	6.0	7.0	8.0
		<i>Mg</i>	<i>Mg</i>	<i>Mg</i>	<i>Mg</i>	<i>Mg</i>
32	Normal.....	0.55	2.00	2.35	1.00	0.55
33	{do.....	.45	1.90	2.16	.93	.66
	{Control.....	0	0	0	0	0
36	Fed Paris green.....	.79	2.21	2.48	.88	.65
37	{do.....	.68	1.92	2.17	.73	.48
	{Control.....	0	0	0	0	0
38	Fed lead arsenate.....	.85	2.46	2.59	.61	.58
39	{do.....	.78	1.91	2.25	.79	.68
	{Control.....	0	0	0	0	0

* In terms of milligrams of glucose per cubic centimeter of incubated mixture.

LIPASE

For determining the activity and presence of lipase, the titration of the free acid resulting from the hydrolysis of an olive-oil emulsion was used. In a 100 c c beaker, 25 c c of a 1 per cent neutral olive-oil emulsion and 12 c c of tissue suspension were placed, with thymol as a preservative. The controls contained the same quantity of boiled tissue suspension. The beakers were left in the dark at room temperature, and at intervals measured portions of the digest were removed and tested for acidity by titration with 0.05N sodium hydroxide. In Table 5 the acidity is indicated in cubic centimeters of 0.05N sodium hydroxide per 5 c c of the incubated mixture. These experiments demonstrated that lipase was present in the mid-gut.

PROTEOLYTIC ENZYMES

To follow the course of digestion by proteolytic enzymes several methods may be utilized. After preliminary work a 5 per cent gelatin solution, into which were incorporated several crystals of thymol as a preservative, was adopted as a substrate. The experiments covered a range of pH 2.0 to 8.0 with convenient pH intervals in order to test for the presence of both peptic and tryptic enzymes.

TABLE 5.—*Digestion of a neutral olive-oil emulsion by lipase obtained from a mid-gut tissue suspension prepared from normal and arsenic-fed potato beetles*

Experiment No.	Treatment of beetles	Acidity * after—						
		4 hours	8 hours	16 hours	24 hours	36 hours	48 hours	Total
		<i>C c</i>	<i>C c</i>	<i>C c</i>	<i>C c</i>	<i>C c</i>	<i>C c</i>	<i>C c</i>
45	{Normal.....	0.32	0.45	0.75	1.10	1.50	1.80	5.92
	{Control.....	.15	.18	.20	.30	.35	.40	1.58
47	{Fed Paris green.....	.37	.40	.65	.85	1.05	1.20	4.52
	{Control.....	.11	.20	.25	.35	.35	.45	1.71
48	{Fed lead arsenate.....	.30	.50	.70	.95	1.20	1.55	5.20
	{Control.....	.15	.25	.30	.40	.40	.45	1.95

* In cubic centimeters of 0.05N sodium hydroxide per 5 cc of digest.

LIQUEFACTION OF GELATIN

Into each of a series of test tubes 10 c c of the gelatin solution previously adjusted to pH 7 with sodium carbonate, 2 c c of tissue suspension, and 2 c c of buffer solution of a desired hydrogen-ion concentration were accurately measured. A similar series containing boiled tissue suspension served as controls. The solutions were covered with a layer of toluene and were placed in a water bath at 37° C. At intervals the tubes were removed and immersed in an ice bath at 0° to 5° for 30 minutes, and the extent of digestion was estimated by observing the degree of liquefaction of the gelatin as described by Dernby (5), where the number 0 indicates no liquefaction and 6 indicates complete liquefaction. (Table 6.) The hydrogen-ion concentration of the contents of the tube reaching the highest number during the shortest interval of time indicated the optimum hydrogen-ion concentration at which digestion occurred.

The results of experiment 51 indicate two optima, one after 48 hours' digestion at pH 5, the other after 55 hours' digestion at pH 7. Although the pH range of activity in this case extended into both the acid and alkaline sides of neutrality—that is, from pH 4 to pH 8—it is quite evident, from what is known of the pH range at which pepsin is active, that in experiments 51 and 53 pepsin was not the enzyme concerned, for none of the contents of the tubes in the range of pH 2 to 4 gave any indication of digestion. The proteolytic enzymes present in the regurgitated liquid were active in the same range (pH 4 to 8), the optimum occurring in the shortest interval of time, namely, 24 hours at pH 5 to 8.

TABLE 6.—*Digestion of gelatin by proteolytic enzymes obtained from a mid-gut tissue suspension prepared from normal and arsenic-fed potato beetles*

Experiment No.	Treatment of beetles	Age of tissue suspension	Period of digestion	Extent of liquefaction * at pH of—									
				2.0	2.6	3.0	4.0	5.0	6.0	6.6	7.0	7.6	8.0
		Days	Hours										
51	Normal.....	7	24	0	0	0	1	1	1	1	0	0	0
			48	0	0	0	1	6	5	2	4	0	0
			55	0	0	0	2	6	5	3	6	4	0
			72	0	0	0	2	6	6	5.5	6	6	5.5
53	Control.....	1/2	72	0	0	0	0	0	0	0	0	0	0
			6	0	0	0	3	6	6	6	6	6	6
55	Normal.....	1	6	0	0	0	0	0	0	0	0	0	0
			24	0	0	0	0	0	0	0	0	0	0
56	Control.....	21	24	0	0	0	0	1	1	1	1.5	2	0
			48	0	0	0	0	1.5	3.5	4.5	5.5	5.5	5
66	Normal.....	2	48	0	0	0	0	0	0	0	0	0	0
			24	0	0	0	1	4	5	5	6	6	5
60	Fed Paris green.....	1	48	0	0	0	1	5.5	6	6	6	6	5.5
			72	0	0	0	1.5	6	6	6	6	6	6
69	Fed lead arsenate.....	2	72	0	0	0	0	0	0	0	0	0	0
			24	0	0	0	0	0	0.5	2	3	4	5
71	Control.....	7	48	0	0	0	5.5	6	6	6	6	6	6
			48	0	0	0	0	0	0	0	0	0	0
71	Fed lead arsenate.....	7	24	0	0	0	0	0	0	0	2	2	0
			48	0	0	0	0	0	0	1	2	2	0
71	Control.....	96	48	0	0	0	0	1.5	1.5	4	4.5	5.5	1.5
			72	0	0	0	0	2	4	4.5	5.5	6	4
71	Control.....	96	96	0	0	0	0	0	0	0	0	0	0
			96	0	0	0	0	0	0	0	0	0	0

* Complete liquefaction equals 6.

† Regurgitated liquid was used instead of tissue suspension.

The influence of the age of a tissue suspension on its activity was determined in the same manner as described above. A few typical results selected from the experiments are given in Table 6.

With a tissue suspension 1 day old (experiment 55) complete digestion took place in 24 hours at pH 4 to 8. With the same tissue suspension 2 days old (experiment 66) there was practically complete digestion after 48 hours at pH 5 to 8. With the same tissue suspension 1 week old (experiment 51) complete digestion occurred in 48 hours only at pH 5, but after 72 hours digestion was practically complete in every tube from pH 5 to pH 8. With the same tissue suspension 3 weeks old (experiment 56) practically complete digestion occurred only at pH 7 to pH 8 in 48 hours. In other experiments not shown in Table 6, performed with tissue suspensions 1 and 6 hours old, digestion in the tubes extended more and more into the acid range. Complete digestion at the pH range 2 to 4, however, was never attained. Hence the conclusion was drawn from these experiments that pepticlike enzymes are not present in the potato beetle.

Experiments were also performed with other proteins as substrates—namely, blood fibrin, casein, and albumin. All these experiments were quite conclusive in indicating that pepticlike enzymes are not present in the potato beetle.

FORMOL TITRATION METHOD

The digestion of gelatin by proteolytic enzymes was also tested by Sorensen's (16) formol titration method. This method depends on the formation of methylene compounds by the combination of formaldehyde with amino groups of amino acids. The acid carboxyl groups can then be estimated by titration with barium hydroxide or sodium hydroxide. The greater the concentration of the amino acids produced by the hydrolysis of a protein the greater the concentration of carboxyl and the greater the acidity following the addition of neutral formaldehyde solution. Thus the increased acidity is a measure of the degree of hydrolysis.

The formol titration was used in an experiment in which the hydrogen-ion concentration of the digest was adjusted at the beginning by the addition of hydrochloric acid or sodium hydroxide as was necessary. The change in acidity during digestion was measured in terms of cubic centimeters of 0.01N sodium hydroxide titrated to pH 8.4. A buffered solution of pH 8.4 was used as a standard, with phenolphthalein as an indicator. The effect of the addition of neutral formaldehyde solution after the titration of the free acidity was to render the solution again acid, and by titration the formol acidity was ascertained, as indicated in Table 7.

By selecting from this table data obtained during digestion at pH 5 and 7, which were the optima attained in the digestion of gelatin, it is noted that at pH 5 the free acidity rises progressively during the course of digestion, whereas the formol acidity does not. In the digestion at pH 7 the process is reversed, the free acidity remaining practically constant during the progress of digestion whereas the formol acidity rises considerably in the latter part of the period of digestion.

TABLE 7.—Formol titration during digestion of gelatin by proteolytic enzymes of the potato beetle carried on at different hydrogen-ion concentrations

pH	Free acidity * after—										Formol acidity * after—									
	4 hours	8 hours	14 hours	24 hours	30 hours	46 hours	52 hours	66 hours	Average	4 hours	8 hours	14 hours	24 hours	30 hours	46 hours	52 hours	66 hours	Average		
1.5	Cc	Cc	Cc	Cc	Cc	Cc	Cc	Cc	Cc	Cc	Cc	Cc	Cc	Cc	Cc	Cc	Cc	Cc		
1.5	5.6	5.5	5.1	5.5	5.2	5.1	5.3	5.4	5.35	0.8	0.8	0.9	0.9	0.9	0.9	0.9	0.9	0.88		
2.0	4.0	4.0	3.9	3.8	3.8	4.0	4.1	4.1	3.96	.8	.8	.9	.9	.8	.8	.8	.9	.84		
2.6	3.9	3.9	3.7	3.7	3.5	3.5	3.8	3.8	3.73	.8	.9	.9	.9	.9	.8	.8	.9	.80		
3.0	2.5	2.2	2.3	2.1	2.1	2.2	2.3	2.2	2.24	.8	.9	.9	.9	.9	.9	.9	.9	.89		
4.0	1.8	1.8	1.8	1.9	1.8	1.8	1.9	1.9	1.84	.9	.9	1.0	.9	.9	1.0	1.0	1.0	.95		
5.0	1.0	1.1	1.2	1.3	1.4	1.5	1.5	1.5	1.31	.9	1.0	1.0	1.0	1.0	1.1	1.2	1.2	1.05		
6.0	.5	.6	.5	.6	.8	.7	.8	.8	.66	1.0	1.0	1.1	1.0	1.1	1.2	1.4	1.6	1.17		
6.6	.5	.4	.5	.6	.5	.6	.7	.6	.55	1.0	.8	.9	1.0	1.0	1.0	1.0	1.2	1.01		
7.0	.4	.5	.5	.5	.5	.5	.5	.6	.50	.9	1.0	1.0	1.0	1.1	1.2	2.0	2.1	1.20		
7.6	.4	.2	.3	.4	.4	.5	.7	.4	.40	.9	1.0	.9	.8	.9	1.1	1.6	1.9	1.14		
8.0	.3	.2	.2	.4	.4	.4	.3	.6	.35	.9	1.0	.9	1.0	.8	1.1	1.1	1.2	1.00		

* Acidity expressed as cubic centimeters of 0.01N sodium hydroxide.

EREPSIN

Erepsin was determined by the method described by Swingle (17) and was found in the mid-gut and regurgitated liquid; none was found in the fore-gut or hind-gut. It is quite likely that the enzyme detected by this method does not differ from the one shown to be active in the liquefaction of gelatin.

THE EFFECT OF ARSENICALS ON THE ACTIVITY OF DIGESTIVE ENZYMES OF THE COLORADO POTATO BEETLE

METHODS OF INVESTIGATION

In the experiments to detect the effect of arsenicals on the activity of digestive enzymes, the insects were first placed on foliage previously sprayed with Paris green or lead arsenate in the proportion usually employed in field tests, namely, 2 pounds of the arsenical to 50 gallons of water. The insects selected for dissection were still alive and were taken from leaves that had been partly consumed. It is not definitely known whether all the insects used in the experiments had actually fed on poisoned foliage. However, it is surmised from their abnormal behavior that they had probably ingested some of the poison as they fed on the foliage. It is quite likely that the quantity of the poison consumed by different individuals varied considerably, and that some beetles may not have fed at all during these experiments, even when they were taken from leaves showing feeding areas. But the percentage of nonpoisoned beetles that may have been used in the dissections must have been very small indeed and would not have influenced the results to any great extent. Usually the beetles that fed on poisoned foliage ceased to feed and remained alive in some instances for several days. The dissection of such beetles revealed considerable food in the digestive tract. The food was removed, and the dissected parts were immersed in several changes of 0.2 per cent sodium chloride solution and distilled water before they were finally ground with sand and made into tissue suspensions.

Since the experiments with normal insects indicated that the enzymes were present only in the mid-gut, the preparation of tissue

suspensions for the following experiments was confined to the mid-gut. Two suspensions were prepared, a glycerol suspension and a water suspension, and the subsequent tests were carried out in the manner described under the treatment of the normal tissue suspension.

EXPERIMENTAL RESULTS

AMYLASE

The effect of arsenicals on the activity of amylase is shown in Table 1, experiments 5 and 6. In the case of beetles that fed on foliage sprayed with Paris green, a retardation of the initial activity of this enzyme was indicated in the range of pH 6.0 to 7.0, and complete digestion of starch was not attained even in 115 minutes. In the case of beetles that fed on foliage sprayed with lead arsenate, no retardation in the initial activity of this enzyme was observed, and, though complete digestion was not attained in 115 minutes, a higher degree of digestion than in the previous experiment was reached.

LACTASE

Experiments 15 to 18 in Table 2 indicate no effect of arsenicals on the activity of lactase.

MALTASE

The experiments with maltase, shown in Table 3, also disclose no influence of arsenicals on the activity of maltase.

INVERTASE

Table 4 demonstrates the activity of invertase of both the normal and the arsenic-fed beetles. Experiments 36 to 39 reveal no influence of arsenic on the activity of invertase.

LIPASE

Experiments 47 and 48 in Table 5 disclose no definite influence of arsenicals on the activity of lipase.

PROTEOLYTIC ENZYMES

The effect of arsenicals on the activity of proteolytic enzymes is shown in Table 6 by experiments 60, 69, and 71. Experiment 60 shows no influence of arsenic on the activity of tryptic enzymes when insects were fed foliage sprayed with Paris green. Similarly, experiment 69 shows no effect of lead arsenate on the activity of tryptic digestion; and experiment 71, in which the tissue suspension was 1 week old before testing, indicates no digestion whatever during the first 24 hours. Practically complete digestion occurred only in certain tubes after 96 hours, a condition similar to that recorded in the case of beetles that were not fed arsenicals, as shown by experiments 51 and 56.

In other experiments not indicated in Table 6, the beetles were injected with Paris green or lead arsenate suspensions. In both cases complete inhibition of the activity of the proteolytic enzymes occurred, no digestion being recorded after 5 days' incubation of the mixtures prepared. The greater inhibition of the activity of the proteolytic enzymes in the latter experiments was probably produced by the larger doses administered by injection.

DISCUSSION

The failure of moderate doses of Paris green and lead arsenate to inhibit markedly the activity of the digestive enzymes of the potato beetle is not surprising in view of the results reported in the literature (1, 2, 3, 11, 12, 13, 14, 15) on the effects of arsenicals on the activity of commercial enzymes. It seems to be generally agreed that small doses of arsenic acid, arsenious acid, atoxyl, ammonium arsenite, and methyl arsenoxide have no inhibiting effect on the activity of any of the enzymes investigated and in some instances even exert an activating influence. Stronger concentrations of arsenical solutions (from 2 to 10 per cent) caused retardation and complete inhibition of the activity of enzymes that attack disaccharides and proteins.

In the present experiments potato beetles were poisoned in the same way as in earlier experiments on the effect of arsenicals on the respiratory metabolism of insects (6). It can therefore be concluded that arsenicals exert a more profound effect on cellular respiration than on the activity of digestive enzymes of the potato beetle. Consequently the writer is inclined to believe that the toxic action of arsenicals on insects can not be explained, even in part, by their effect on digestive enzymes.

SUMMARY OF RESULTS

With few exceptions, in the normal insect all the enzymes studied, namely, amylase, lactase, invertase, lipase, and the proteolytic enzymes, were found only in the mid-gut and regurgitated liquid. In the exceptional cases it is believed that parts of tissue from the mid-gut may have adhered to the fore-gut and hind-gut during the process of dissection. This assumption is based on the fact that only a few experiments indicated digestion in either the fore-gut or hind-gut and that the digestive activity of the enzymes in these instances was extremely slight.

It is quite clear from experiments herein reported that arsenic does not influence the digestive enzymes of potato beetles fed on sprayed foliage to the extent of inhibiting their normal activity. In general, when they are fed on foliage sprayed with an arsenical, there is but little retardation of the activity of amylase and tryptic enzymes, and none at all of enzymes active in the digestion of disaccharides and fats. However, the injection of arsenical suspensions directly into the insect mouth results in complete inhibition of the activity of the proteolytic enzymes.

LITERATURE CITED

- (1) CHITTENDEN, R. H., and ALLEN, S. E.
1885. INFLUENCE OF VARIOUS INORGANIC AND ALKALOID SALTS ON THE PROTEOLYTIC ACTION OF PEPSIN-HYDROCHLORIC ACID. Conn. Acad. Arts and Sci. Trans. (1885-1888) 7:84-107.
- (2) ——— and CUMMINS, G. W.
1885. INFLUENCE OF VARIOUS THERAPEUTIC AND TOXIC SUBSTANCES ON THE PROTEOLYTIC ACTION OF THE PANCREATIC FERMENTS. Conn. Acad. Arts and Sci. Trans. (1885-1888) 7:[108]-124.
- (3) ——— and PAINTER, H. M.
1885. INFLUENCE OF CERTAIN THERAPEUTIC AND TOXIC AGENTS ON THE AMYLOLYTIC ACTION OF SALIVA. Conn. Acad. Arts and Sci. Trans. (1885-1888) 7:[60]-83.

- (4) CLARK, W. M.
1922. THE DETERMINATION OF HYDROGEN IONS; AN ELEMENTARY TREATISE ON THE HYDROGEN ELECTRODE, INDICATOR AND SUPPLEMENTARY METHODS, WITH AN INDEXED BIBLIOGRAPHY ON APPLICATIONS. Ed. 2, 480 p., illus. Baltimore.
- (5) DERNBY, K. G.
1918. A STUDY OF AUTOLYSIS OF ANIMAL TISSUES. *Jour. Biol. Chem.* 35:179-219, illus.
- (6) FINK, D. E.
1926. PHYSIOLOGICAL STUDIES OF THE EFFECT OF ARSENICALS ON THE RESPIRATORY METABOLISM OF INSECTS. *Jour. Agr. Research* 33:993-1007, illus.
- (7) ———
1927. A MICRO METHOD FOR ESTIMATING THE RELATIVE DISTRIBUTION OF GLUTATHIONE IN INSECTS. *Science (n. s.)* 65:143-145.
- (8) ———
1927. IS GLUTATHIONE THE ARSENIC RECEPTOR IN INSECTS? *Jour. Econ. Ent.* 20:794-801, illus.
- (9) ———
1927. THE APPLICATION OF STUDIES IN HYDROGEN ION CONCENTRATION TO ENTOMOLOGICAL RESEARCH. *Ann. Ent. Soc. Amer.* 20:503-512, illus.
- (10) PARFENTJEV, J. A., and DEVRIENT, W.
1930. ÜBER DIE WIRKUNG DES ARSENS AUF DEN GASSTOFFWECHSEL BEI INSEKTEN. *Biochem. Ztschr.* 217:[368]-377.
- (11) PINCUSOHN, L.
1908. BEEINFLUSSUNG VON FERMENTEN DURCH KOLLOIDE. I. WIRKUNG VON ANORGANISCHEN KOLLOIDEN AUF PEPSIN. *Biochem. Ztschr.* 8:[387]-398.
- (12) RONA, P., AIRILA, Y., and LASNITZKI, A.
1922. BEITRÄGE ZUM STUDIUM DER GIFTWIRKUNG. ÜBER DIE KOMBINIERTE WIRKUNG DES CHININS UND DER NARKOTICA AUF INVERTASE UND ÜBER DIE WIRKUNG VON ARSENVERBINDUNGEN AUF MALTASE UND α -METHYLGLUCOSIDASE. *Biochem. Ztschr.* 130:[582]-591, illus.
- (13) SCHAFFER, F., and BÖHM, R.
1872. ÜBER DEN EINFLUSS DES ARSENS AUF DIE WIRKUNG DER UNGEFORMTEN FERMENTE. *Jahresber. Fortschr. Thierchemie* 2:363-365.
- (14) SMORODINZEW, J. A., and RIABOUSHCHINSKY, N. P.
1924. ZUR FRAGE NACH DEM EINFLUSS VON ARSEN- UND ANTIMONVERBINDUNGEN AUF DIE FERMENTATIVEN FUNKTIONEN DES ORGANISMUS. II. MITTEILUNG: DER EINFLUSS EINIGER ARSEN- UND ANTIMONPRÄPARATE AUF DAS PEPSIN. *Biochem. Ztschr.* 144:26-30.
- (15) ——— and RIABOUSHCHINSKY, N. P.
1926. ZUR FRAGE DES EINFLUSSES VON ARSEN- UND ANTIMONVERBINDUNGEN AUF DIE FERMENTATIVEN FUNKTIONEN DES ORGANISMUS. III. MITTEILUNG: ÄNDERT SICH DIE WASSERSTOFFZAHL DES MILIEUS WÄHREND DER VERDAUUNG DES CASEINS DURCH PEPSIN IN ANWESENHEIT VON ARSEN- UND ANTIMONVERBINDUNGEN? *Biochem. Ztschr.* 168:[73]-76.
- (16) SÖRENSEN, S. P. L.
1908. ENZYMSTUDIEN. *Biochem. Ztschr.* 7:[45]-101.
- (17) SWINGLE, H. S.
1925. DIGESTIVE ENZYMES OF AN INSECT. *Ohio Jour. Sci.* 25:209-218.
- (18) VOEGTLIN, C., DYER, H. A., and LEONARD, C. S.
1925. ON THE SPECIFICITY OF THE SO-CALLED ARSENIC RECEPTOR IN THE HIGHER ANIMALS. *Jour. Pharmacol. and Expt. Ther.* 25:297-307.

A STUDY OF SEVERAL FACTORS IN THE SEPARATION OF SERUM FROM BOTTLED CREAM¹

By G. MALCOLM TROUT, *Research Assistant in Dairying*, and J. C. McCAN, *Graduate Research Assistant in Dairying, Michigan Agricultural Experiment Station*

INTRODUCTION

The separation of a layer of skim milk, or serum, at the bottom of the bottle of cream is a defect of considerable importance to the market-cream distributor. The consumer naturally attributes the presence of this skim milk, even when an insignificant layer appears in whipping cream containing as much as 40 per cent butterfat, to a very low fat content.

The greater portion of market cream is sold as table, or coffee, cream. Practically all of this grade of cream is standardized by the distributor to contain approximately 20 per cent butterfat. Any correlation by the consumer of the skim-milk layer with the fat content is especially undesirable because, as the distributor knows, many factors other than the fat content of the cream affect the volume of this serum layer.

The frequency of this cream defect and the attendant complaints from customers, combined with a lack of definite information bearing directly on the problem, seemed to make a study of some of the factors incident to this problem desirable.

REVIEW OF LITERATURE

A survey of the literature reveals many data on the creaming of milk, but although very closely associated, few data have been collected which bear directly on the separation of free serum from bottled cream. Many theories are current concerning the best methods of processing cream in the plant to reduce the prominence of this layer. Milk-plant practices in processing cream are based largely upon results obtained from cream-line studies of market milk and general observation in commercial plants.

The settling out of a layer of skim milk from bottled cream, or rather the "creaming off" of cream, seems to be the result of a greater degree of enmeshing and orientation of the individual fat globules and fat clusters. As early as 1899 Farrington and Russell (2)² observed that the cream volume on milk was reduced after the milk had been creamed for 48 hours. The reduction of the cream volume of milk following its initial formation was later observed by Dahlberg and Marquardt (1), Hammer (3), Judkin and Downs (4), Martin and Combs (5), Trout (6), and Whitaker et al. (8).

The fat content of gravity cream has been observed (6) to range from 11.0 to 28.5 per cent, depending upon the nature and previous treatment of the milk and upon the conditions of creaming. Following the introduction of improved machinery and new methods, the

¹ Received for publication Jan. 28, 1932; issued October, 1932. Journal article No. 87 (n. s.) from the Michigan Agricultural Experiment Station.

² Reference is made by number (italic) to Literature Cited, p. 500.

standardization of table cream to approximately 20 per cent butterfat became the common practice of market-cream distributors. This seemingly low fat content of the standardized cream in comparison with that of some gravity cream secured under certain conditions has had its part in causing the cream distributor to homogenize his product, although the principal reason perhaps for homogenizing was to increase the viscosity of the cream, which gave it a heavier body and thus a richer appearance. However, beyond certain pressures of homogenization, as pointed out by Webb and Holm (7), feathering of the cream resulted when homogenized cream was added to coffee. The procedure in many smaller milk plants in processing market table cream is that only of standardizing the fat content of the cream, and the homogenization process is entirely eliminated. This cream, when bottled and held at storage temperatures ranging from 35° to 40° F. for 24 to 48 hours, often revealed a serum layer, which was objectionable to the consumer for the reasons previously mentioned.

SCOPE OF THE INVESTIGATION

Few data are available that show the effect of the temperature at which the milk is skimmed upon the separation of free serum from the resulting cream, either before or after standardization. Likewise, there is little information regarding the effect of the heat treatment of milk previous to separation on the appearance of the serum layer. Practically all the effects noted as resulting from certain milk-plant operations have been concerned with the creaming of milk rather than with the stability of the cream once it has formed. The object of this study was to determine the effects on the stability of cream of various factors, such as heat treatment of milk and cream, standardization, viscolization, and the addition of gelatin and salts.

METHODS

Cream was obtained from mixed milk containing from 3.4 to 3.8 per cent butterfat. This milk was secured each day from the same 300-gallon pasteurizer in the college creamery after it had been filled with milk delivered by the same group of milk patrons.

For the major part of the experimental work, for which only small lots of cream were required, the milk was separated in the experimental laboratory with a small electrically driven separator. Preliminary runs were made to determine the proper screw setting to yield, at the various temperatures, cream having approximately the desired percentage of butterfat. One gallon of milk was allowed to run through the machine in order to temper the bowl before the samples of cream and skim milk were saved. Larger quantities of cream were obtained by separating milk with a larger separator in the college creamery. In one series of experiments, so far as possible, a 20 per cent cream was separated, while in another series approximately a 40 per cent cream was secured, which was later standardized back to 20 per cent butterfat by being combined with the skim milk previously separated.

The milk, both fresh and stored, was skimmed at temperatures extending from 60° to 145° F. before pasteurization, at 145° for 30 minutes, and at similar temperatures following the pasteurization exposure. One series of experiments was conducted in which ex-

posures prior to separation of 145° for 30 minutes, 160° for 15 minutes, and 180° momentarily following the 160° exposure were used.

Four lots of raw cream which had been separated at 90° to 110° F. and standardized to 20 per cent butterfat were exposed for 30 minutes to temperatures of 145°, 150°, 155°, and 160°, respectively. Similarly standardized cream secured from pasteurized milk was exposed to the same degree of temperature for the same length of time. The holding or vat method of pasteurization was used.

Some of the cooling was done in the pasteurizing vats, some by placing the cream or milk in 1-gallon cans set in water and gently stirred at intervals, and some over a surface cooler. The product was cooled to 60° F. for standardization or for taking the samples.

When trials were made with gelatin a high grade of commercial gelatin was used. The gelatin was mixed with 1 pint of cold water and heated in a tank of hot water until completely dissolved. The dissolved gelatin was then added to a 60-pound lot of milk in the pasteurizer at 120° F. as the milk was being heated to 145° for separation.

Various percentages of sodium carbonate, calcium chloride, monocalcium phosphate, dicalcium phosphate, and sodium citrate were added to the cream. With the exception of dicalcium phosphate, which was not so soluble, these salts were made up so that 5 c c of the solution would give 0.1 per cent of the salt when added to a 500 c c sample of cream. Dicalcium phosphate was added as a saturated solution.

The condensed milk used was a well-known commercial brand of sterilized unsweetened evaporated milk which had been concentrated approximately in the ratio of 2 to 1. The milk powder used, testing 95 per cent solids, was made by the spray process and was obtained from a local manufacturer. The casein used was a commercial brand of soluble food casein.

Samples of the cream were taken in order to determine the volume of the serum layer appearing in the cream, and also to observe the distinctness or attention-attracting power of this layer, as evidenced (1) by the sharpness of the line of demarcation between the layers, and (2) by the difference in the appearance of the cream and the serum layers as a result of the completeness of separation of the fat. It was believed at first that both observations could be made by setting the cream in standard 100 c c graduated cylinders which were used to determine the volume of the layer of free serum. Preliminary runs, however, showed that the distinctness of the serum layer was always much greater in bottles than in the cylinders. Samples were, therefore, taken both in cylinders and in ½-pint cream bottles; in the cylinders to determine the volume of the serum layer and in the bottles to observe the distinctness of this layer. The samples were taken after the cream had been cooled to approximately 60° F. The cylinders and bottles were then cooled in ice water to 40° and set aside for creaming.

The milk and cream were stored in the cold-storage room of the creamery, which ranged in temperature from 35° to 40° F. All samples set for creaming were placed in this room, with the exception of a few samples in that part of the experiment concerned with the effect of the temperature of creaming. In this study samples were set in ice water in the cold room at 32°, in an electric refrigerator held at a constant temperature between 39° and 41°, in cold running water main-

tained at 56°, and in a culture oven kept at 68° to 70°. Two drops of 40 per cent formaldehyde were added to each pint of cream as a preservative, after preliminary study had showed that the addition of this preservative in considerably larger quantities had no noticeable effect on cream rising.

All butterfat tests were made according to the Babcock method.

The observations of creaming were made after 48 hours, except when it was necessary to study the results after 72 hours of creaming. Considerable difficulty was encountered in this part of the work, especially in observing the creaming in the cylinders, where the separation of the layers was far less distinct than in the bottles. Sudan III was used in an attempt to make more accurate and more reliable readings possible but without success. A microscope light was tried in order to standardize the source of light for these observations. Preliminary work, however, showed that the best source of light was indirect sunlight, which was used in making all observations.

The volume of the serum layer was recorded in terms of percentage. The degree of distinctness of the skim-milk layer was recorded by means of letters, as follows: A=wholly distinct; B=very distinct; C=fairly distinct; D=not very distinct; E=barely observable; F=no layer, but showing a different appearance between the lower and upper portions due to some separation of skim milk; and G=no evident separation.

The results of the work recorded represent averages in the majority of cases of at least six trials of every factor studied; in some cases the results of only four trials, while in others the averages of many more trials are recorded. A total of 126 different lots of cream was prepared, treated, and observed.

EFFECT OF THE HEAT TREATMENT OF THE MILK PRIOR TO SEPARATION

FRESH MILK

The temperature of the fresh milk at the time of separation was found to influence both the amount and the distinctness of the skim-milk layer, as shown in Tables 1 and 2. Separation of the milk at the receiving temperature, 55° to 65° F., gave a deeper serum layer than did separation at any of the higher temperatures. This difference was very evident in the bottled cream as well as in the cylinders. Temperatures of separation ranging from 90° to 180° were found to have little influence on the amount of the serum; although there was considerable difference in the distinctness of the layer. Separation at the receiving temperature, 55° to 65°, or at 90°, gave a considerably more distinct serum layer than did separation at 120° or above. At 145° the layer was more distinct than at 120°. This difference, however, was only slight and might have been due to the effect of heat on the milk during the time required for the separation process, since pasteurization at 145° for 30 minutes, as will be shown in the consideration of the effect of pasteurization, was found to increase the distinctness of the layer.

TABLE 1.—*Effect of heat treatment of fresh milk, prior to separation, on the volume and distinctness of the serum layer when low-test cream was secured*

[Average of six trials]

Lot No.	Heat treatment	Fat in cream	Serum layer	Distinctness of serum layer
		<i>Per cent</i>	<i>Per cent</i>	
1	Heated to 90° F. (check).....	20.8	1.83	B—
2	Heated to 120° F.	19.5	2.04	D—
3	Heated to 145° F.	20.6	2.0	C—
4	Heated to 145° F. for 30 minutes, then cooled to 120°.....	21.2	2.1	C+
5	Heated to 145° F. for 30 minutes, then cooled to 90°.....	22.0	2.0	C+

TABLE 2.—*Effect of heat treatment of fresh milk, prior to separation, on the volume and distinctness of the serum layer, when high-test cream was secured and standardized back to approximately 20 per cent butterfat*

[Average of six trials]

Lot No.	Temperature of separation	Fat in cream secured	Fat in standardized cream	Serum layer	Distinctness of serum layer
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
1	55-65° F.	50.4	20.4	2.83	B
2	90° F.	38.5	19.9	2.2	B+
3	120° F.	42.3	20.1	2.38	D—
4	145° F.	41.8	20.6	2.0	D
5	145° for 30 minutes.....	44.4	20.2	2.0	C—

The influence of the temperature of separation at 90° and 120° F. varied, depending upon whether the milk was being heated up to those temperatures or was being cooled from a higher temperature. Pasteurizing milk at 145° for 30 minutes, followed by subsequent cooling to 120° and 90° for separation, gave no appreciable difference in the distinctness or volume of the serum layer at the two temperatures. However, when the temperature of the milk was raised from the temperature at which it was received at the plant to 90° and to 120°, separation at 90° gave a far more distinct cream line.

STORED MILK

The results of storing milk for 24 to 48 hours at 35° to 40° F. and separating the cream after slowly and carefully warming the milk to the various temperatures are given in Table 3. The temperature of separation of old milk was found to have little effect on the amount of the serum layer. The results indicate that the time of storing the milk at low temperatures had little influence upon the volume of the serum layer as compared to that obtained from fresh milk, except at 60°. A comparison of the data in Tables 2 and 3 shows that when old stored milk is separated at that temperature the serum layer is reduced to 2.07 per cent, whereas in the case of fresh milk it is 2.83 per cent. The distinctness of the serum layers varied somewhat according to the temperature of separation.

When the temperature of the old stored milk was raised to 80° to 90° F. before separation the cream yielded showed a much more distinct serum layer than when the separating was done at 60°. Separating old milk at 80°, 90°, 120°, and 145° revealed that there was little

difference between separating old milk and separating fresh milk at similar temperatures in regard to the distinctness of the serum layer. As with separating fresh milk, separating old milk at 120° gave a much less distinct layer than separating it at 80° or 90°. Raising the temperature from 120° to 145° before separating increased the distinctness to a slight extent.

TABLE 3.—*Effect of heat treatment of stored milk, prior to separation, on the volume and distinctness of the serum layer*

[Average of eight trials]

Lot No.	Temperature of separation	Fat in cream secured	Fat in standardized cream	Serum layer	Distinctness of serum layer
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
1	60° F.....	24.5	20.5	2.07	C
2	80° F.....	20.2	20.2	1.95	A—
3	90° F.....	20.4	19.8	2.14	B+
4	120° F.....	19.3	19.3	1.84	E+
5	145° F.....	20.3	20.3	2.0	D+

PASTEURIZATION

The results of separating milk after heating it to 145° F. momentarily, after pasteurizing it at 145° for 30 minutes, after pasteurizing it at 145° and cooling it to 120°, after pasteurizing it at 160° for 15 minutes, and after raising the temperature of the milk pasteurized at 160° to 180° indicate (Table 4) that, with the exception of the milk pasteurized at 160° for 15 minutes, the pasteurization exposure had little influence upon the amount of the serum layer. When the milk was pasteurized at 160° for 15 minutes and skimmed at that temperature, a smaller volume of serum appeared in the resulting cream than appeared in that secured at any other temperature of pasteurization. These results are shown in Tables 1, 2, and 4.

TABLE 4.—*Effect of high temperature of fresh milk, prior to separation, on the volume and distinctness of the serum layer*

[Average of seven trials]

Lot No.	Heat treatment	Fat in cream	Serum layer	Distinctness of serum layer
		<i>Per cent</i>	<i>Per cent</i>	
1	Heated to 145° F. (check).....	19.8	2.13	D
2	Heated to 160° F. for 15 minutes.....	20.4	1.76	C—
3	Heated to 160° F. for 15 minutes, then raised to 180° for separation..	20.7	2.11	C—

Heating milk to 145° F. and separating it immediately resulted in a less distinct serum layer than was obtained in the check sample secured at 90°. Pasteurizing the milk at 145° for 30 minutes made the serum layer more distinct than that secured when the milk was merely heated to 145°, but the layer was considerably less distinct than that secured in the check sample at 90°. Pasteurizing the milk at 160° for 15 minutes, or at this same exposure and then raising the temperature to 180° momentarily, before separating, gave a slightly

more distinct serum layer than was obtained when the milk was heated only to 145° momentarily. (Table 4.)

EFFECT OF HEAT TREATMENT OF THE CREAM

The results obtained by pasteurizing raw standardized cream and repasteurizing pasteurized standardized cream are given in Table 5. Pasteurizing raw cream at each of the 5-degree intervals from 145° to 160° F. for 30 minutes yielded a serum layer of less volume but of greater distinctness than that in the raw cream. The different temperatures used between 145° and 160° apparently had no marked influence either on the volume or on the distinctness of the layer. One temperature was as satisfactory as the other. Approximately a 1.9 per cent serum layer appeared in the cream obtained from the pasteurized cream, as compared with a 2.5 per cent serum layer in the cream obtained from the raw cream.

TABLE 5.—*Effect of different temperatures of pasteurization of raw and pasteurized standardized cream on the volume and distinctness of the serum layer*

[Average of four trials]

Temperature maintained for 30 minutes	Lots in which cream and skim milk used for standardization were obtained from raw milk				Lots in which cream and skim milk used for standardization were obtained from milk pasteurized at 145° F. for 30 minutes			
	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
Unpasteurized(check)	47.5	20.0	2.5	D—	38.8	20.0	1.85	B—
145° F.	47.5	20.0	1.95	C+	38.8	20.0	1.95	B—
150° F.	47.5	20.0	1.98	C+				
155° F.	47.5	20.0	1.9	C+				
160° F.	47.5	20.0	1.85	C+	38.8	20.0	1.8	B—

Repasteurizing standardized pasteurized cream at 145° F., or at 160°, for 30 minutes apparently had no appreciable effect either on the volume or on the distinctness of the serum layer, as shown by the results in Table 5. This cream was secured by mixing pasteurized whole milk with cream from pasteurized milk.

EFFECT OF VARIATIONS OF THE MANY FACTORS CLOSELY ASSOCIATED WITH STANDARDIZATION

HIGH-TEMPERATURE PASTEURIZATION OF THE MILK VERSUS HIGH-TEMPERATURE PASTEURIZATION OF THE CREAM USED IN STANDARDIZATION

Lots of raw cream testing 38.5 per cent butterfat were standardized with raw skim milk and with skim milk pasteurized at 170° F. for 20 minutes. Also lots of raw skim milk were used to standardize raw high-fat-content cream and similar cream pasteurized at 170° for 20 minutes.

TABLE 6.—*Effect of high temperature of pasteurization of either the skim milk or cream, or both, before and after standardization*

[Average of six trials]

Product and heat treatment ^a	Lots in which cream and skim milk used for standardization were obtained from raw milk				Lots in which cream and skim milk used for standardization were obtained from milk pasteurized at 145° F. for 30 minutes			
	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer
Cream pasteurized before standardization	<i>Per cent</i> 38.5	<i>Per cent</i> 20.7	<i>Per cent</i> 2.3	C	<i>Per cent</i> 44.6	<i>Per cent</i> 20.0	<i>Per cent</i> 2.47	D
Skim milk pasteurized before standardization	^b 38.5	^b 20.7	2.45	B	^b 44.6	^b 20.0	2.20	D
Cream pasteurized after standardization	38.5	19.8	4.3	C—	44.6	20.25	6.17	D—

^a 170° F. for 20 minutes.^b Percentage of fat in the cream.

The data presented in Table 6 show little difference in the volume of the serum layers appearing in either of the standardized creams of which one or the other of the products used was heated to a high temperature. It will be observed that the detrimental effect was very great when the cream was pasteurized at 170° F. for 20 minutes after standardization. Similar results were obtained when the cream and skim milk were secured from pasteurized milk rather than from raw milk.

When the skim milk was secured from raw whole milk and was pasteurized before being used in standardizing the raw cream the distinctness of the serum layer was much greater.

STANDARDIZING PASTEURIZED CREAM WITH MILK PROCESSED IN VARIOUS WAYS

High-test cream was standardized with raw whole milk, with pasteurized whole milk, and with skim milk from pasteurized milk. The normal exposure of 145° F. for 30 minutes was employed. It will be observed from the data in Table 7 that no appreciable differences occurred in the volume of the serum layer from the cream standardized with the different milks, either raw or pasteurized. The distinctness of the layer, however, was very slightly less in every case when raw whole milk was used in the standardization than when pasteurized whole milk or pasteurized skim milk was used.

TABLE 7.—*Effect of standardizing pasteurized cream with raw whole milk, with pasteurized whole milk, and with skim milk from pasteurized milk*

[Average of five trials]

Lot No.	Kind of milk used	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer
		<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
1	Raw whole	45.2	20.7	1.88	C—
2	Pasteurized whole	45.2	20.3	1.98	C+
3	Skim milk from pasteurized milk	45.2	20.4	2.08	C+

SEPARATING HIGH-TEST CREAM AND STANDARDIZING BACK VERSUS SEPARATING LOW-TEST CREAM

The opinion seems to be current among milk-plant operators that high-test cream standardized back to a low fat content gives a greater serum layer than cream separated to contain a low fat content, which requires less standardizing. The data obtained in this experiment as presented in Table 8 show a tendency toward a higher volume of serum layers in the high-test cream standardized back to approximately 20 per cent than in the cream separated as near 20 per cent as possible and not standardized. However, with many difficult readings due to the indistinctness of the serum layers in the cylinders in this experiment, definite conclusions can not be drawn. Apparently there was no significant difference in the distinctness of the serum layer in the standardized and unstandardized cream.

TABLE 8.—*Effect of standardizing high-fat-content cream back to approximately 20 per cent butterfat with the corresponding skim milk obtained during the separation as compared with results on a 20 per cent cream as separated*

[Average of six trials]

Temperature of separation	Cream standardized back to about 20 per cent fat content				Cream of about 20 per cent fat content as separated			
	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer	Fat before standardization	Fat after standardization	Serum layer	Distinctness
90° F.....	Per cent 38.5	Per cent 19.9	Per cent 2.2	B+	Per cent 20.8	Per cent 20.8	Per cent 1.83	B—
120° F.....	42.3	20.1	2.38	D—	19.5	19.5	2.04	D
145° F.....	41.8	20.0	2.0	D	20.6	20.6	2.0	C—

STANDARDIZING WITH WHOLE MILK VERSUS STANDARDIZING WITH SKIM MILK

The data secured when raw cream was standardized with raw whole or with raw skim milk presented in Table 9, show little difference either in the amount of the serum layer or in its distinctness due to the factor of richness of the standardizing media. On the other hand, when pasteurized cream was standardized with pasteurized whole milk or with pasteurized skim milk, a serum layer of less volume appeared in the cream (Table 7) than when raw cream was standardized with raw whole milk or with raw skim milk (Table 9). This appears to substantiate the former results obtained showing that pasteurization decreased the serum layer.

TABLE 9.—*Effect of standardizing raw cream with raw whole milk versus standardizing raw cream with raw skim milk*

[Average of six trials]

Milk used	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer
Whole.....	Per cent 46.0	Per cent 20.3	Per cent 2.3	C
Skim.....	46.0	20.8	2.5	D

STANDARDIZATION BEFORE VERSUS STANDARDIZATION AFTER PASTEURIZATION

In studying the effect of standardization before as compared with standardization after pasteurization, raw cream was standardized with raw whole milk. This product was then pasteurized at the ordinary exposures. The results were compared with those obtained by standardizing previously pasteurized high-test cream with pasteurized milk. It will be observed from the data shown in Table 10 that the serum layer was somewhat greater in volume when the standardizing was done prior to pasteurization than when it was carried out after pasteurization. There seemed to be no marked difference in distinctness as a result of the two methods.

TABLE 10.—*Effect of standardizing cream before versus standardizing it after pasteurization*

[A verage of five trials]

When standardized	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	
Before pasteurization.....	45.2	20.4	1.9	B—
After pasteurization.....	42.2	20.9	1.5	B—

TEMPERATURE OF STANDARDIZATION

Since the temperature of separation and pasteurization found to affect the distinctness and volume of the serum layer, it was thought possible that the temperature of the milk and of the cream at the time of mixing in standardization might have an influence on the volume and distinctness of the serum layer. However, the results of four trials, in which the milk and cream were mixed hot and cold in all possible combinations, showed that neither the temperature of the cream nor that of the milk had any appreciable effect on the amount or on the distinctness of the serum layer when raw cream was standardized with raw skim milk.

STANDARDIZING CREAM FRESH VERSUS STANDARDIZING IT AFTER 24 HOURS IN STORAGE

The data secured indicate that a slightly greater serum layer formed when the cream was standardized fresh. However, standardization after storing gave a layer of considerably greater distinctness, as will be observed from the data presented in Table 11.

TABLE 11.—*Effect of standardizing cream fresh versus standardizing cream after storing 24 hours*

[A verage of seven trials]

Kind of cream standardized	Fat before standardization	Fat after standardization	Serum layer after—		Distinctness of serum layer after—	
			48 hours	120 hours	48 hours	120 hours
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>		
Fresh.....	42.7	19.8	1.91	4.29	C—	F+
Stored 24 hours at 35° F.....	42.7	19.9	1.75	3.71	B—	C—

EFFECT OF MISCELLANEOUS CONDITIONS

AGITATION OF CREAM AFTER STORING

The cream was stored in the cold-storage room at 35° to 40° F. for 24 hours, after which it was agitated violently, to determine whether such agitation, after the fat was in a hardened condition, had any influence on the serum layer. The data secured are presented in Table 12. It was found that agitation of pasteurized cream after it had been stored 24 hours very slightly increased the volume of the serum layer. Furthermore, agitation of the stored cream caused the formation of a more distinct layer.

TABLE 12.—*Effect of agitating standardized cream which had been stored 24 hours at a low temperature*

[Average of six trials]

Agitation of cream	Temperature of separation	Temperature of pasteurization	Fat before standardization	Fat after standardization	Serum layer	Distinctness of serum layer
	° F.	° F.	Per cent	Per cent	Per cent	
Unagitated (check)	90-100	145	44.4	20.1	2.1	C—
Agitated after 24 hours at 35° F.	90-100	145	44.4	20.1	2.2	B—

METHOD OF COOLING

Since considerable data are found in the literature showing greater creaming when the milk is cooled rapidly, a study of this factor was made with pasteurized cream to determine the degree of separation of free serum. The rapid cooling was done over a surface cooler, while the slow cooling was accomplished by occasionally gently stirring the cream, which was set in a vat of cold water. The data obtained, as presented in Table 13, indicate that the speed of cooling pasteurized cream had little effect either upon the volume or upon the distinctness of the resulting serum layer.

TABLE 13.—*Effect of the method of standardizing and cooling cream*

When standardized	Trials	Temperature of separation	Fat before standardization	Fat after standardization	Serum layer when—		Distinctness of serum layer when—	
					Surface cooled	Vat cooled	Surface cooled	Vat cooled
	Number	° F.	Per cent	Per cent	Per cent	Per cent		
Before pasteurization	5	90-100	45.2	20.4	1.88	1.94	B—	C—
After pasteurization	5	90-100	45.2	20.9	1.66	1.64	C+	C
After cooling following pasteurization	4	90-100	44.9	20.5	1.90	1.83	C	C—

TEMPERATURE OF CREAMING

The data presented in Table 14 show no appreciable difference either in the volume or in the distinctness of the serum layer when the creaming was attempted in ice water as compared with that secured at the cooling-room temperatures of 35° to 40° F. Later work, after an accurate, temperature-controlled, electric refrigerator had been obtained and the higher temperature constantly main-

tained at 39° to 41°, gave data which seemed to indicate that at ice-water temperature a slightly smaller serum layer was formed than formed at 39° to 41°. At higher temperatures the layers were even less distinct. The actual volume of the layers could not be observed in the cylinders as the line of demarcation was indistinct, but the volume of the serum layers in the bottles was larger at 56° and at 70°, the volume at the latter temperature being the greater.

TABLE 14.—Effect of the temperature of creaming

Temperature of separation	Trials	Fat before standardization	Fat after standardization	Serum layer when creaming was done in or at—					Distinctness of serum layer when creaming was done in or at—				
				Ice water	35°-40° F.	39°-41° F.	56° F.	70° F.	Ice water	35°-40° F.	39°-41° F.	56° F.	70° F.
90° to 100° F..	Number	Per cent	Per cent										
145° F.-----	5	45.5 36.0	20.5 20.0	1.83 1.86	1.8	2.08	(*)	(*)	C B	C	B-	D-	F+

* Too indistinct for accurate reading.

EFFECT OF DURATION OF TIME OF CREAMING

The data secured indicate that as the duration of time of creaming was extended the volume of the serum layer increased. The distinctness of the serum layer increased for the first 72 hours; after that time its distinctness diminished. It was difficult to determine the volume of the serum layer, especially after 96 hours, as the serum layers in the cylinders became less distinct. The distinctness seemed to increase for some 48 to 72 hours and then gradually decreased. Many observations in the study of the various factors

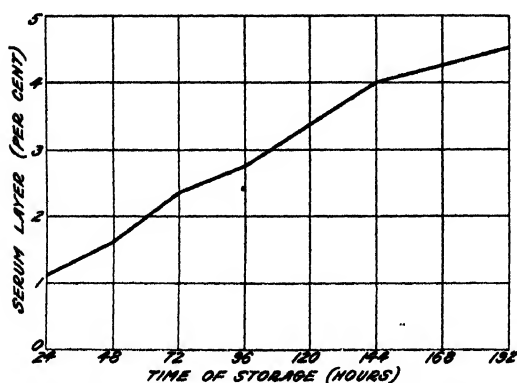


FIGURE 1.—Increase of serum layer formed in cream during storage at 37° F.

were made at the end of 96 hours, in addition to the observations at the end of 48 hours, as a check on the first readings. It was always very evident that the layer was less distinct after 96 hours. In studying the effect of storing the milk and cream a day before standardization, in which a comparison of the stored, standardized sample was made with the check sample set raw on the fifth and sixth days of creaming, respectively, it was found in every case that the serum layers were considerably less distinct after creaming had been going on for five or six days than after creaming had been going on only 48 hours. The serum layers were slightly more than twice as great in volume after the longer period of creaming. (Fig. 1.)

EFFECT OF VISCOLIZATION
TEMPERATURE AND PRESSURE

Raw standardized cream was viscolized at 145° F. and at 160° at pressures of 500, 1,000, 1,500, 2,000, and 2,500 pounds per square inch. The data presented in Table 15 show that low pressures reduced the creaming as compared with the unviscolized cream, and that the volume of the serum layer was reduced by increases in pressure until the layer was entirely eliminated. Since free serum was sometimes noted in the bottles when there was no apparent separation of the cream in the cylinders this was tabulated in the results. A "small layer" indicates that an appreciable amount of separation took place and would probably be noticed by the cream buyer. A "trace" indicates a very small layer often noticed only after careful observation of the samples, and would probably never be evident to the consumer. It was found that at low pressures at which the serum layer was not entirely eliminated, the small remaining layer was more distinct than in the check, the unviscolized samples. Viscolizing at 160° as compared with viscolizing at 145° generally gave a less distinct serum layer at low pressures, when a serum layer appeared. The layer was also usually entirely eliminated at a lower pressure when the milk was processed at 160° rather than at 145°, thus indicating that viscolization was more efficient at high than at low temperatures. Viscolizing raw cream at 1,500 pounds pressure per square inch at 145° or 1,000 pounds pressure at 160° ordinarily eliminated the serum layer entirely or reduced the layer to such an extent that it would probably have passed unnoticed by the consumer.

TABLE 15.—Effect of viscolizing raw cream originally containing 45.88 per cent fat but standardized at 20 per cent at various pressures and temperatures

[Average of four trials]

Pressure used in viscolizing (pounds per square inch)	Serum layer when cream was processed at 145° F.		Distinctness of serum layer when cream was processed at 145° F.		Serum layer when cream was processed at 160° F.		Distinctness of serum layer when cream was processed at 160° F.	
	Unviscolized	Viscolized	Unviscolized	Viscolized	Unviscolized	Viscolized	Unviscolized	Viscolized
	Per cent	Per cent			Per cent	Per cent		
0	2.1		C—		1.75		C—	
500		(a)		C—		(a)		E—
1,000		(b)		C—		(a)		F
1,500		(c)		C—		None.		G
2,000		None.		F		None.		G
2,500		None.		G		None.		G

^a Too indistinct for accurate reading.

^b Small layer.

VISCOLIZING HIGH-TEST CREAM

Attempts to eliminate the serum layer by viscolizing high-test cream before standardizing it with unviscolized milk proved unsuccessful. However, the serum layer was always considerably reduced in volume by this treatment. On the other hand, this method gave a much more distinct serum layer, thus overcoming the advantages of the reduced volume.

VISCOLIZING ONLY A PORTION OF THE CREAM

By mixing 10 per cent of cream, standardized to 20 per cent fat and viscolized at 2,500 pounds pressure with unviscolized cream testing 20 per cent fat, the volume of the serum layer was decreased from 2.1 to 1.18 per cent, or reduced approximately 44 per cent. The treatment had no significant influence on the distinctness of the layer as compared with that in the check samples which had not been viscolized.

EFFECT OF THE ADDITION OF VARIOUS SALTS

Sodium carbonate, sodium citrate, calcium chloride, dicalcium phosphate, and monocalcium phosphate were added in varying percentages to cream in order to determine the effect of these salts on the separation of free serum. The cream used was separated from milk at temperatures between 90° and 100° F., was standardized from 44.5 per cent butterfat to 20 per cent butterfat, and was pasteurized at 145° for 30 minutes. After the salts were added, the samples were held at 35° to 40° for 48 hours before observations were made. The results are given in Table 16. The addition of 0.1 per cent sodium carbonate considerably increased the volume and made the layer slightly more distinct than in the check samples. The addition of 0.3 and 0.5 per cent decreased the volume as compared with that when 0.1 per cent was used, but a partial precipitation of the curd took place, as demonstrated by the clear whey which was left in the bottom instead of the usual serum.

TABLE 16.—*Effect of the addition of various salts to pasteurized cream containing 20 per cent of butterfat*

[Four trials in each test]

SALT ADDED BY WEIGHT, DRY

Salts used	Serum layer when indicated percentage of salt was added				Distinctness of serum layer when indicated percentage of salt was added			
	None	0.1	0.3	0.5	None	0.1	0.3	0.5
Sodium carbonate.....	1.78	3.6	2.28	1.95	C—	B—	A+	A+
Calcium chloride.....	1.78	1.75	1.75	1.75	C—	C—	D	D—
Dicalcium phosphate.....	1.78	1.95	1.85	1.84	C—	C—	C—	C—
Sodium citrate.....	1.78	1.65	3.33	3.45	C—	C—	A+	A+

SALT ADDED AS CUBIC CENTIMETERS OF SATURATED SOLUTION

	None	5	15	25	None	5	15	25
Monocalcium phosphate.....	1.78	2.33	2.25	8.25	C—	C—	D	D

CUBIC CENTIMETERS OF WATER ADDED, CHECKS

	None	5	15	25	None	5	15	25
Check samples.....	1.78	1.75	1.85	1.93	C—	C—	C—	C—

The addition of calcium chloride in the various amounts apparently had no marked influence on the volume of the serum layer, but the addition of 0.3 and 0.5 per cent resulted in the serum layer becoming less distinct.

Dicalcium phosphate in the amounts added apparently had no measurable effect either on the size or on the distinctness of the serum layer.

The addition of one-tenth of 1 per cent sodium citrate evidently had no marked effect either on the volume or on the distinctness of the layer, but adding 0.3 and 0.5 per cent markedly increased both the volume and the distinctness of the layer. This may have been due to a partial precipitation of the casein, for the serum was only slightly milky at the bottom. This indicated that the greater portion of the casein had been carried up with the fat.

The addition of 5, 15, and 25 c c of a saturated monocalcium phosphate solution increased the volume of the serum, especially in case of the most concentrated sample. This salt also reduced the distinctness of the layer to a slight extent, particularly when 15 c c and 25 c c additions of the solution were made. The 15 c c concentration caused a slight precipitation of the casein and the 25 c c concentration caused a considerable precipitation which was noticed during the agitation of the samples at the time the solution was added.

EFFECT OF THE ADDITION OF GELATIN, EVAPORATED MILK, SKIM-MILK POWDER, AND SOLUBLE FOOD CASEIN

Instead of being added to the cream, the gelatin was added to the milk prior to separation. The cream secured from the various lots was then compared after being stored at 35° to 40° F. for 30 minutes. Except when gelatin was added, the cream used in these experiments was separated from milk at 90° to 100°, then standardized from 45 per cent butterfat to 20.5 per cent butterfat. Before the addition of the various substances, the standardized cream was pasteurized at 145° for 30 minutes. After the substances had been introduced and mixed the samples were stored at 35° to 40° for 48 hours before observations were made.

GELATIN

Some very interesting results were obtained by adding small amounts of gelatin to the milk prior to separation, as shown in Table 17. It was found that by adding 0.1 per cent of gelatin, the amount of the serum layer was increased slightly, although accurate readings were difficult as this amount of the colloid gave a layer of considerably less distinctness than those in the check samples containing no gelatin. The addition of 0.2 per cent gelatin, however, caused a reduction of approximately 40 per cent in the volume of the serum layer as compared to that in the check sample, but the layer was much more distinct. Except in one trial in which a trace of a serum layer remained, it was found that adding 0.3 per cent of gelatin practically eliminated and adding 0.4 per cent of gelatin always entirely eliminated the layer.

TABLE 17.—*Effect of the addition of gelatin to raw milk prior to separation*

[Average of six trials]

Gelatin added to milk	Fat in cream	Serum layer	Distinctness of serum layer	Gelatin added to milk	Fat in cream	Serum layer	Distinctness of serum layer
<i>Per cent</i> None. 0.1 .2	<i>Per cent</i> 20.5 21.4 21.9	<i>Per cent</i> 2.37 2.66 1.42	C+ D- A	<i>Per cent</i> 0.3 .4	<i>Per cent</i> 22.5 23.3	<i>Per cent</i> None. None.	G G

EVAPORATED MILK AND SKIM-MILK POWDER

Since it was understood that concentrated milk solids are frequently added to cream in some commercial plants in an attempt to control the serum layer in bottled cream, trials were made to determine the effect of adding evaporated milk and skim-milk powder. These were added at the rate of 0.5, 1.0, 2.0, and 5.0 per cent. The results are shown in Table 18. One-half of 1 per cent of evaporated milk had no marked influence either on the volume or on the distinctness of the serum layer. When larger amounts of evaporated milk were added, the serum layer decreased markedly both in volume and in distinctness. The addition of 5 per cent of evaporated milk eliminated the layer entirely.

The results fail to show that skim-milk powder in any of the percentages added had any influence on the size of the layer, although accurate observations were difficult when the larger amounts were added. No separation was evident in the cylinders when 5 per cent of the powder was added, but a layer was discernible in the bottled cream. It was apparent from the results that, although from 1 to 5 per cent of skim-milk powder was an aid in lessening the distinctness of the serum layer, the addition of skim-milk powder did not eliminate the serum layer entirely, as did evaporated milk when added at the rate of 5 per cent.

TABLE 18.—*Effect of the addition of evaporated whole milk, skim-milk powder, and casein to pasteurized cream containing 20.5 per cent butterfat*

Substance added	Trials	Serum layer when indicated percentage additions were made					Distinctness of serum layer when indicated percentage additions were made				
		0	0.5	1.0	2.0	5.0	0	0.5	1.0	2.0	5.0
Evaporated whole milk.....	<i>Number</i> 2	2.0	2.0	1.5	1.5	None.	C-	C-	D	E	G
Skim-milk powder.....	2	2.0	2.0	2.0	2.0	Trace.	C-	C-	C-	D	E
Casein.....	4	1.75				3.88	C-				C+

CASEIN

The results of four trials in adding 0.5 per cent of casein indicated that casein added in this amount increased the volume of the serum layer. The check samples had on an average a 1.75 per cent layer, whereas in the samples to which casein had been added the layer averaged 3.88 per cent. There also seemed to be a tendency for the layer to be more distinct, although this effect was not pronounced.

SUMMARY AND CONCLUSIONS

From the data secured it appears that the separation of serum from bottled cream is a defect common to cream processed in the plant by the methods tested other than homogenizing or adding various substances. Separating the milk at 120° F. seemed to prevent, in part at least, the formation of a distinct serum layer, although the volume of serum layer remained practically the same irrespective of the temperature of separation. On the other hand, pasteurizing milk at 160° for 15 minutes prior to separation decreased the volume of the serum layer but had little effect upon the distinctness. Storing milk prior to separation caused no appreciable difference either in the volume or in the distinctness of the serum layer.

It appears that pasteurization alone even at as high a temperature as 160° for 30 minutes fails to prevent the formation of a distinct serum layer. However, pasteurizing raw cream at 145°, 150°, 155°, or 160° F. for 30 minutes decreased the volume and increased the distinctness of the serum layer, there being no marked difference from the results obtained at any exposure. Agitating stored pasteurized cream had no significant influence upon the volume of the serum layer, but caused it to be more distinct. The speed of cooling pasteurized cream apparently had little effect upon the volume or upon the distinctness of the serum layer.

The opinion has been current that this serum layer in bottled cream results from separating a high-fat-percentage cream and then standardizing it back to the desired richness. It seems from the data obtained that neither the method nor the heat treatment of the medium used in standardization materially reduces the volume of serum layer or lessens the intensity of its distinctness. Standardizing the cream following pasteurization appears to be desirable in eliminating, in part, the formation of a layer of serum but has little effect on its distinctness.

The length of time cream is held in storage subsequent to bottling is an important factor in the formation of the serum layer. Cream held in storage longer than 48 hours after being bottled almost invariably shows an undesirable serum layer. The volume of the serum layer increased gradually at a decreasing rate as the cream was held. The distinctness seemed to increase for some 48 to 72 hours and then gradually decreased. An increase in the temperature of creaming above 32° F. increased the volume and decreased the distinctness of the serum layer.

Viscolization at 160° F. and a pressure of 1,500 pounds per square inch appears to be equally as effective in preventing the formation of a layer of serum as 2,500 pounds pressure at 145° F. Mixing 10 per cent of cream viscolized at 145° F. with unviscolized cream reduced the layer about 44 per cent. The distinctness was not influenced to any marked extent.

The salts added gave various results. Dicalcium phosphate apparently had no influence either upon the volume or upon the distinctness of the layer. Calcium chloride, while having no apparent effect upon the volume, decreased the distinctness to some extent. The other salts added, sodium citrate, sodium carbonate, and monocalcium phosphate, apparently did not influence "creaming off" until amounts were added that partly or totally precipitated the casein.

The addition of 0.3 per cent gelatin to the milk prior to separation appears to prevent entirely the formation of a layer of serum in the cream secured. Likewise, the addition of 5 per cent evaporated whole milk to the cream entirely eliminated the serum layer. No separation was evident in the cylinders when 5 per cent skim-milk powder was added, but a layer was discernible in the bottled cream. It was apparent that although 1 to 5 per cent skim-milk powder was an aid in lessening the distinctness of the serum layer, it did not eliminate the layer entirely as when 5 per cent evaporated whole milk was added. These additions to milk and cream, although in some cases attaining the desired purpose, should be considered as adulterations, and as such are not to be recommended.

The appearance of a serum layer seems to be a defect common to most cream processed without homogenization or additions. By bottling the cream not longer than 36 to 48 hours before final distribution the formation of the serum layer is usually kept at a minimum. Pasteurizing the milk intended for skimming at 160° F. for 15 minutes, separating at 120° F., and standardizing to the desired percentage with pasteurized whole milk seems to yield a cream in which the smallest serum layer of least distinctness forms.

LITERATURE CITED

- (1) DAHLBERG, A. C., and MARQUARDT, J. C.
1929. THE CREAMING OF RAW AND PASTEURIZED MILK. N. Y. State Agr. Expt. Sta. Tech. Bul. 157, 80 p., illus.
- (2) FARRINGTON, E. H., and RUSSELL, H. L.
1899. PASTEURIZATION OF MILK AND CREAM AT 140° F. Wis. Agr. Expt. Sta. Ann. Rpt. 16: 129-139, illus.
- (3) HAMMER, B. W.
1916. STUDIES ON THE CREAMING ABILITY OF MILK. Iowa Agr. Expt. Sta. Research Bul. 31, 91 p., illus.
- (4) JUDKINS, H. F., and DOWNS, P. A.
1918. STUDIES IN PROCESSING MILK. Conn. Storrs Agr. Expt. Sta. Bul. 99, p. [449]—470.
- (5) MARTIN, W. H., and COMBS, W. B.
1924. THE EFFECT OF MILK PLANT OPERATIONS ON THE AMOUNT OF CREAM RISING ON MILK. Jour. Dairy Sci. 7: 197-204, illus.
- (6) TROUT, G. M.
1930. CREAM LINE STUDIES IN MARKET MILK. W. Va. Agr. Expt. Sta. Bul. 229, 20 p., illus.
- (7) WEBB, B. H., and HOLM, G. E.
1928. THE HEAT STABILITY AND FEATHERING OF SWEET CREAM, AS AFFECTED BY DIFFERENT HOMOGENIZATION PRESSURES AND DIFFERENT TEMPERATURES OF FOREWARMING. Jour. Dairy Sci. 11: 243-257, illus.
- (8) WHITTAKER, H. A., ARCHIBALD, R. W., SHEVE, L., and CLEMENT, C. E.
1925. EFFECT OF VARIOUS FACTORS ON THE CREAMING ABILITY OF MARKET MILK. U. S. Dept. Agr. Bul. 1344, 24 p., illus.

INHERITANCE OF RESISTANCE TO BUNT, *TILLETIA TRITICI*, IN HYBRIDS OF WHITE FEDERATION AND ODESSA WHEAT¹

By FRED N. BRIGGS

Assistant Agronomist, California Agricultural Experiment Station

INTRODUCTION

The inheritance of resistance to bunt, *Tilletia tritici* (Bjerk.) Wint., has been reported for hybrids between susceptible varieties of wheat and the resistant varieties Martin (1),² Hussar (1, 2), White Odessa (3), and Banner Berkeley (4). The present paper deals with the inheritance of resistance to bunt in hybrids between susceptible White Federation and resistant Odessa wheats. Data are also included from Martin × White Odessa which were not available for the earlier publication on White Odessa (3).

METHODS AND MATERIALS

The parental material and hybrid populations were grown in the field at University Farm, Davis, Calif. The methods of handling and the inoculum used have been described in previous publications (1, 2, 3, 4).³ This bunt has been designated as physiologic race III of *Tilletia tritici*, by Reed (7).

The resistant parent was obtained in 1919 under the name of Gold-coin but was later identified as Odessa, by J. A. Clark, Division of Cereal Crops and Diseases, United States Department of Agriculture.

That Odessa is very resistant as compared with White Federation may be seen in Table 1.

TABLE 1.—Annual percentage of bunt infection in the parent wheat varieties

Variety	Percentage of bunted plants in—							
	1920	1921	1922	1927	1928	1929	1930	1931
Odessa.....	0.6	0	0.7	0	0	0	0	0
Martin.....	0	0	0	0	0	0	0	0
White Federation.....	88.7	51.6	58.3	66.6	68.9	78.6	62.2	44.9

It is not known just why Odessa produced a little bunt during 1920 and 1922 but has been bunt free in other years when grown under similar conditions and inoculated with bunt from the same original source. Similar results were obtained with White Odessa (3) and Banner Berkeley wheats (4). Such small amounts of bunt are of little importance. Martin has been completely resistant during the entire period.

¹ Received for publication Dec. 21, 1931; issued October, 1932.

² Reference is made by number (italic) to Literature Cited, p. 505.

³ BRIGGS, F. N. INHERITANCE OF RESISTANCE TO BUNT, *TILLETIA TRITICI*, IN CROSSES OF WHITE FEDERATION WITH TURKEY 1668 AND TURKEY 8065. Unpublished manuscript.

EXPERIMENTAL RESULTS

Some of the F₂ seeds of all crosses were treated with copper carbonate to protect them from bunt infection in order to grow seed for the F₃ generation. The rest of the F₂ seeds were inoculated with bunt and grown the following year along with the F₃ rows.

F₂ data do not permit a satisfactory Mendelian analysis because some susceptible plants usually escape infection. Also heterozygous resistant and homozygous resistant plants occasionally may become infected. F₂ data do indicate, however, the percentage of bunt to expect in F₃ rows of the same genotype. Accordingly, the results are given in Table 2.

TABLE 2.—Percentage of bunted plants in the parents and F_2 of the crosses named when grown in the field at Davis, Calif.

Parent or cross	Year grown	Plants grown	Plants hunted	
			Number	Per cent
Odessa.....	1930	412	0	0
Martin.....	1930	141	0	0
White Federation.....	1930	546	334	61.2
F ₁ White Federation X Odessa.....	1930	1, 161	175	15.1
F ₁ Martin X Odessa.....	1930	658	0	0
White Odessa.....	1931	143	0	0
Martin.....	1931	150	0	0
White Federation.....	1931	648	291	44.9
F ₁ Martin X White Odessa.....	1931	359	0	0

There was an average of 15.1 per cent of bunted plants in the F_2 of White Federation \times Odessa, which is considerably less than the 25 per cent expected on the basis of a single dominant factor for resistance. Martin \times White Federation produced 17.2 per cent of bunted plants in F_2 (1). At that time it was shown that enough susceptible plants had escaped infection to bring this figure into satisfactory agreement with the 25 per cent expected. The F_2 data, then, indicate that Odessa differs from White Federation in a single dominant factor for resistance to bunt, and that heterozygous F_3 rows should contain an average of about 15 per cent of bunt.

TABLE 3.—*Distribution of parent and F₃ rows of the crosses named into 5 per cent classes for bunt infection, when grown at Davis, Calif.*

[illegible]

The F_2 of Martin \times Odessa was free from bunt, indicating that Odessa has the same factor for resistance to bunt as Martin. F_2 data of Martin \times White Odessa indicate that White Odessa also has the Martin factor.

Obviously, the classification of F_2 plants on the basis of the behavior of their progeny in F_3 rows is more reliable than classification in F_2 . F_3 rows contained from 30 to 60 plants. These data are recorded in Table 3.

The rows having from 0 to just less than 5 per cent of bunted plants were separated into those with no bunted plants and those with some but less than 5 per cent of bunted plants, because of the special interest in the former. The nature of the distribution may be seen more readily in Figure 1. The numbers of rows under the three modes are in close agreement with the 1:2:1 ratio. As in the crosses with Martin, White Odessa, and Banner Berkeley the first minimum fell at

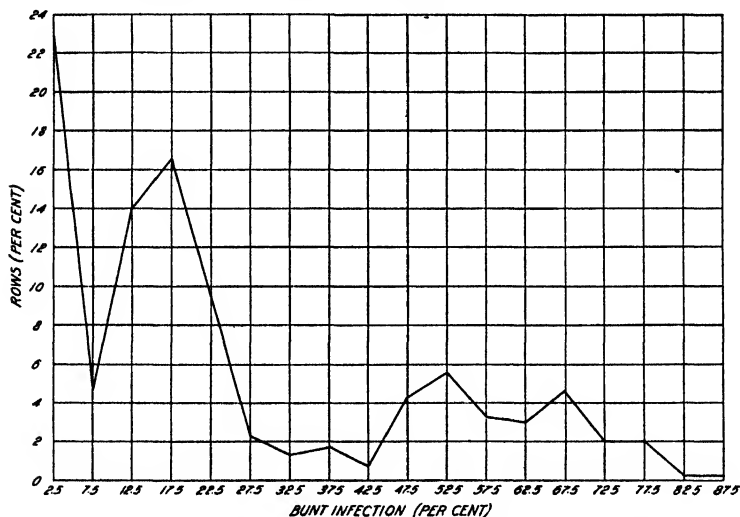


FIGURE 1.—Distribution of F_3 rows of the cross White Federation \times Odessa into 5 per cent classes of bunt infection

7.5 per cent of bunted plants. The division between the heterozygous and the susceptible rows is not so sharp but has been somewhere near 50 per cent of bunt infection, falling at 42.5 in this case. As divided by these minima there were 76 resistant, 148 heterozygous, and 78 susceptible rows where 75.5, 151.0, and 75.5, respectively, were expected. The susceptible rows had an average of 60.1 per cent of bunt while 12 rows of White Federation averaged 62.2 per cent. The heterozygous rows averaged 18.5 per cent under the same conditions under which 30 F_2 rows produced 15.1 per cent of bunted plants.

No doubt some rows are classified incorrectly by accepting these minima as the points of division between phenotypes. Duplicate plantings were made of 53 F_3 rows and their classification was compared, using the minima established by the entire F_3 as the points of division between phenotypes. The second planting was made in the same nursery but at some distance from the first. The results are shown in Table 4.

TABLE 4.—Percentage of bunted plants in duplicate plantings of F_3 row of White Federation \times Odessa

Row No.	Percentage of bunt		Row No.	Percentage of bunt		Row No.	Percentage of bunt		Row No.	Percentage of bunt	
	Series 1	Series 2		Series 1	Series 2		Series 1	Series 2		Series 1	Series 2
1.....	48.3	67.5	15.....	1.6	0	28.....	54.8	60.3	41.....	20.3	17.5
2.....	0	2.3	16.....	17.2	11.3	29.....	16.7	19.6	42.....	51.2	43.6
3.....	0	0	17.....	14.3	14.8	30.....	0	2.0	43.....	21.0	21.3
4.....	23.1	11.8	18.....	3.9	1.7	31.....	20.0	14.0	44.....	0	0
5.....	66.7	53.2	19.....	47.5	52.5	32.....	19.6	22.8	45.....	58.5	57.4
6.....	33.3	45.3	20.....	54.3	61.8	33.....	17.1	29.6	46.....	0	0
7.....	7.8	20.0	21.....	66.7	53.8	34.....	60.0	54.7	47.....	20.4	9.3
8.....	16.4	13.5	22.....	23.5	11.8	35.....	25.5	23.6	48.....	13.0	29.3
9.....	0	0	23.....	12.7	27.5	36.....	0.0	1.7	49.....	12.8	21.3
10.....	12.3	18.6	24.....	1.7	3.5	37.....	15.6	7.3	50.....	25.0	18.5
11.....	13.7	22.4	25.....	68.4	67.2	38.....	18.2	19.1	51.....	35.5	10.0
12.....	16.7	9.8	26.....	0	0	39.....	0	0	52.....	0	0
13.....	14.1	15.5	27.....	27.5	8.8	40.....	2.4	2.7	53.....	14.3	20.0
14.....	49.1	44.8									

In a number of cases the actual percentages of bunt vary considerably between series 1 and series 2, but in many cases they are in close agreement. The average percentage of bunt in the first series is 21.9 and in the second 21.7. The classification in the second series differs from the first in only two cases. No. 6 changed from heterozygous to susceptible and No. 37 from heterozygous to resistant. A similar experiment was made with 36 F_3 rows of White Federation \times Banner Berkeley. The data are very similar to those in Table 4, even though the first planting was made in 1929 and the duplicate planting in 1930. The classification differed in only one case. No. 28 was classified as heterozygous in 1929, but as resistant with 7.3 per cent of bunt in 1930. These duplicate plantings indicate that the minima indicate the division between phenotypes quite accurately.

The classification of F_2 plants, determined on the basis of the percentage of bunted plants in F_3 rows, shows that Odessa differs from White Federation in one main dominant factor for resistance to bunt. That this factor is identical with the factor in Martin wheat is evidenced by the fact that there was no bunt in the F_2 or 149 F_3 rows of the Martin \times Odessa cross.

In an earlier publication (3) it was shown that White Odessa differed from White Federation in one main dominant factor for resistance to bunt, which was similar in its effect to the Martin factor. The factor for resistance to bunt in White Odessa is identical with the factor in Martin.

DISCUSSION AND SUMMARY

The literature discussed in earlier publications will not be repeated here. Recently, Churchward (5) has reported that Florence differs from Hard Federation in one main factor for resistance to bunt. This is of special interest because Florence is a parent of Ridit, which is very resistant to bunt, and was selected from a cross of Turkey \times Florence, by Gaines (6). The writer⁴ has found that Turkey differs from White Federation in one main factor for resistance. Unless there are some factors in Turkey or Florence which are not apparent

⁴ BRIGGS, F. N. Op. cit. (See footnote 3.)

in the presence of the physiologic forms of bunt used in these experiments, Redit should not contain more than these two factors for resistance to bunt. However, unpublished data from crosses of Florence \times White Federation and Florence \times Big Club, grown at this station, have yielded data which the writer has not been able to analyze satisfactorily for Mendelian ratios. This may indicate the presence of a large number of modifying factors or possibly multiple factors. These points are being investigated. A difference in bunt might give different results. The bunt used at Davis is the one designated as physiologic race III of *Tilletia tritici*, by Reed (7).

Odessa and White Odessa, like Martin and Banner Berkeley, may be designated as *MMhh*. Hussar is of the *MMIIH* constitution. The proper designation for Turkey 1558 and Turkey 3055 is not known at present. They each contain a single factor for resistance which is similar in effect to the second Hussar factor *HH*.

LITERATURE CITED

- (1) BRIGGS, F. N.
1926. INHERITANCE OF RESISTANCE TO BUNT, *TILLETIA TRITICI* (BJERK.) WINTER, IN WHEAT. Jour. Agr. Research 32:973-900, illus.
- (2) ————
1930. INHERITANCE OF THE SECOND FACTOR FOR RESISTANCE TO BUNT, *TILLETIA TRITICI*, IN HUSSAR WHEAT. Jour. Agr. Research 40:225-232, illus.
- (3) ————
1930. INHERITANCE OF RESISTANCE TO BUNT, *TILLETIA TRITICI*, IN WHITE ODESSA WHEAT. Jour. Agr. Research 40:353-359, illus.
- (4) ————
1931. INHERITANCE OF RESISTANCE TO BUNT, *TILLETIA TRITICI*, IN HYBRIDS OF WHITE FEDERATION AND BANNER BERKELEY WHEATS. Jour. Agr. research 42:307-313, illus.
- (5) CHURCHWARD, J. G.
1931. STUDIES IN THE INHERITANCE OF RESISTANCE TO BUNT IN A CROSS BETWEEN FLORENCE \times HARD FEDERATION WHEATS. Roy. Soc. N. S. Wales, Jour. and Proc. 64:298-319, illus.
- (7) GAINES, E. F.
1923. GENETICS OF BUNT RESISTANCE IN WHEAT. Jour. Agr. Research 23:445-480, illus.
- (7) REED, G. M.
1928. PHYSIOLOGIC FACES OF BUNT OF WHEAT. Amer. Jour. Bot. 15:157-170.

THE EFFECT OF ARTIFICIAL DRYING ON THE AVAILABILITY OF THE NUTRIENTS OF ALFALFA HAY¹

By E. B. HART, *Professor of Agricultural Chemistry*, O. L. KLINE, *Assistant in Agricultural Chemistry*, and G. C. HUMPHREY, *Professor of Animal Husbandry*, Wisconsin Agricultural Experiment Station²

INTRODUCTION

Recently there have come into practical use a number of driers designed for the rapid desiccation of large quantities of green plant tissue. Naturally the question of the availability of the nutrients of such dried hays as compared with those of hays cured in the ordinary way becomes important.

One of the first driers used in this country for the desiccation of green plants was the Mason drier. In this machine the period of drying is from 30 to 35 minutes, and the temperature of the heated air as it passes from the furnace to the drying tunnel is 127° to 129° C. Later the Koon drier came into use. A Koon drier, established on the farm of Howard T. Green at Genesee Depot, Wis., was used there experimentally for a number of years. The maximum temperature to which plants were exposed in the process of drying and the time of exposure were definitely measured. The time consumed in the drying process was not more than one minute. The highest temperature recorded for fresh green material was 480° to 535° C. with an exposure of 40 seconds. Where the material had already been partly dried lower temperatures were used at the inlet. Such high temperatures of the drying gases must be used in the evaporation of the water without subjecting the dry matter itself to them. In spite of the high temperatures the hay comes out of the drier retaining a pea-green color. It was not known, however, whether these temperatures had rendered unavailable certain valuable constituents of the plants, and it was to determine the effects of this method of drying on the availability of important nutrients that the experiment herein reported was undertaken.

Studies have been carried out on the effect of the Mason drying process on the vitamin A and D content of alfalfa.³ Russell found that alfalfa cured by the Mason process was seven times as potent in its vitamin A content as field-cured hay, but that it was very low in vitamin D—as would be expected when compared with alfalfa cured in the sun. Hauge and Aitkenhead⁴ also found that alfalfa dried in artificial driers, at a much higher temperature than that used in the Mason drier, was still rich in vitamin A. They say: "Even a temperature as high as the hot flue gas used in one of the machines, or the sterilization temperature of the autoclave, was not destructive." No mention is made of the type of drier used.

¹ Received for publication Mar. 4, 1932; issued October, 1932.

² The writers' thanks are due Howard T. Green, of Brook Hill Farms, Genesee Depot, Wis., for making this investigation possible.

³ RUSSELL, W. C. THE EFFECT OF THE CURING PROCESS UPON THE VITAMIN A AND D CONTENT OF ALFALFA. *Jour. Biol. Chem.* 85: 289-297. 1929.

⁴ HAUGE, S. M., and AITKENHEAD, W. THE EFFECT OF ARTIFICIAL DRYING UPON THE VITAMIN A CONTENT OF ALFALFA. *Jour. Biol. Chem.* 93: 663. 1931.

EXPERIMENTAL PROCEDURE

Three lots of second-growth alfalfa hay, cut from the same field were prepared at the Brook Hill farm. The hays were cured in three different ways: Hay No. 1 was cut on August 1, 1930, and dried immediately in the Koon drier. Hay No. 2 was cut at 2.30 p. m., July 31, raked into a windrow late in the afternoon, hauled just before noon on August 1, and dried in the artificial drier. This hay had lost about 40 per cent of its moisture before it was actually dried in the drier. Hay No. 3 was field cured, cut on August 13, at 3 p. m., raked into windrows on August 14 about 1 p. m., and cocked about 4.30, August 14. This hay was hauled August 20, cut with a silage cutter, and blown into bags.

The hays, cut and bagged, were delivered to the experiment station. All were a rich pea-green color. Studies on the digestible dry matter, digestible protein, and available calcium were made with three high-producing Holstein cows that were in the early part of their third lactation period. The metabolism experiment was begun February 5, 1931. Cow No. 1 freshened January 15, 1931; cow No. 2 freshened December 22, 1930; and cow No. 3 freshened December 20, 1930. All developed into heavy milkers—that is, they produced 50 to 60 pounds of milk daily.

The ration used consisted of 10 pounds of the alfalfa hay, 25 pounds of corn silage, and for every 3 to 3½ pounds of milk 1 pound of a grain mixture made of 59 parts of yellow corn, 25 parts of wheat bran, 15 parts of oil meal, and 1 part of salt.

In the first period of three weeks the animals were fed hay 1 (dried immediately); in the second period of three weeks, hay 2 (partly field cured and then dried in the drier); and in the third period of three weeks, hay 3 (sun cured in the field).

Quantitative collection of the excreta and quantitative collection of the milk, with a complete analysis of income and outgo, particularly in reference to calcium, digestible dry matter, and digestible nitrogen, were made. During the course of the experiment the feed consumption by the animals was complete, except in the ninth week, when cow 2 left some residue.

EXPERIMENTAL DATA

Table 1 gives the record of digestibility of the dry matter. A study of this table indicates that the coefficients of digestibility of the dry matter of the three hays were very much alike. Apparently drying at a high temperature for 40 seconds had in no way impaired the availability of the dry matter of alfalfa for the cow.

TABLE 1.—*Data on digestibility of dry matter in alfalfa dried and cured in different ways*

PERIOD 1—HAY 1 (ALFALFA DRIED IMMEDIATELY)

Week No.	Cow 1				Cow 2				Cow 3			
	Dry matter intake	Dry matter in feces	Difference	Coefficient of digestibility	Dry matter intake	Dry matter in feces	Difference	Coefficient of digestibility	Dry matter intake	Dry matter in feces	Difference	Coefficient of digestibility
1.....	Grams	Grams	Grams	Per ct.	Grams	Grams	Grams	Per ct.	Grams	Grams	Grams	Per ct.
2.....	91, 286	26, 461	64, 825	71. 0	88, 516	25, 558	62, 958	71. 1	91, 286	27, 187	64, 099	70. 2
3.....	91, 286	25, 530	65, 756	72. 0	88, 516	24, 948	63, 568	71. 8	91, 286	28, 063	63, 193	69. 2
.....	91, 286	27, 179	64, 107	70. 2	88, 516	24, 617	63, 899	72. 1	91, 286	28, 745	62, 541	68. 5
Average.....	71. 0	71. 7	69. 3

PERIOD 2—HAY 2 (ALFALFA PARTLY CURED)

4.....	90, 427	26, 713	63, 714	70. 4	87, 662	20, 617	61, 045	69. 6	90, 427	29, 731	60, 696	67. 1
5.....	90, 427	25, 764	64, 664	71. 5	87, 662	23, 976	63, 686	72. 6	90, 427	28, 080	62, 347	68. 9
6.....	90, 427	26, 235	64, 192	71. 0	87, 662	20, 114	61, 548	70. 2	90, 427	27, 044	63, 383	70. 0
Average.....	71. 0	70. 8	68. 7

PERIOD 3—HAY 3—(ALFALFA SUN CURED)

7.....	90, 538	24, 046	65, 892	72. 7	87, 784	25, 017	62, 767	71. 5	90, 538	27, 294	63, 244	69. 8
8.....	90, 538	27, 113	63, 425	70. 0	87, 784	26, 840	60, 944	69. 4	90, 538	28, 482	62, 056	68. 5
9.....	90, 538	26, 309	64, 229	70. 9	87, 784	18, 688	69, 096	(*)	90, 538	28, 189	62, 349	68. 9
Average.....	71. 2	70. 4	69. 1

* Cow off feed.

Table 2 gives a record of the digestible nitrogen. A careful study of this table shows that the protein and other nitrogenous complexes of the hay were not rendered unavailable through the heat treatment of this particular drier. One might interpret the records of cows 1 and 3 as indicating a slightly lower availability of the protein in the hay that was immediately dried as compared with that in the sun-cured hay, but the differences are too slight to be of any real significance. In the case of cow 2 the availability of the nitrogen in the three periods was practically identical.

TABLE 2.—Data on digestibility of nitrogen in alfalfa dried and cured in different ways

PERIOD 1—HAY 1 (ALFALFA DRIED IMMEDIATELY)

Week No.	Cow 1				Cow 2				Cow 3			
	Total nitrogen intake	Nitrogen in feces	Nitrogen digested	Coefficient of digestibility	Total nitrogen intake	Nitrogen in feces	Nitrogen digested	Coefficient of digestibility	Total nitrogen intake	Nitrogen in feces	Nitrogen digested	Coefficient of digestibility
	Grams	Grams	Grams	Per ct.	Grams	Grams	Grams	Per ct.	Grams	Grams	Grams	Per ct.
1.....	2,315.95	642.60	1,673.35	72.2	2,229.85	660.60	1,563.25	70.1	2,315.95	773.14	1,542.81	66.6
2.....	2,315.95	612.70	1,703.25	73.1	2,229.85	631.80	1,598.05	71.6	2,315.95	719.90	1,595.99	68.9
3.....	2,315.95	639.00	1,676.95	72.4	2,229.85	640.80	1,589.05	71.3	2,315.95	827.68	1,488.27	64.3
Average.....				72.6				71.0				66.6

PERIOD 2—HAY 2 (ALFALFA PARTLY CURED)

4.....	2,379.51	598.20	1,781.31	74.9	2,293.41	686.88	1,606.53	70.0	2,379.51	798.75	1,580.76	66.5
5.....	2,379.51	639.00	1,740.51	73.3	2,293.41	642.82	1,650.59	71.9	2,379.51	793.49	1,586.02	66.6
6.....	2,379.51	613.80	1,765.71	74.2	2,293.41	645.84	1,647.57	71.8	2,379.51	685.26	1,694.25	71.2
Average.....				74.1				71.2				68.1

PERIOD 3—HAY 3 (ALFALFA SUN CURED)

7.....	2,363.62	564.60	1,799.02	76.1	2,277.52	652.15	1,625.37	71.3	2,363.62	772.74	1,590.88	67.2
8.....	2,363.62	591.00	1,772.62	75.0	2,277.52	637.81	1,639.71	72.0	2,363.62	773.26	1,590.36	68.9
9.....	2,363.62	552.60	1,811.02	76.0	2,277.52	395.14	1,882.38	(*)	2,363.62	748.50	1,615.12	68.3

* Cow off feed.

In Table 3 are given the records of the calcium metabolism of the cows during the three periods, as well as the weekly milk production. As is usually the case with cows in the early part of lactation, especially liberal producers, these animals are in negative lime balance. The results recorded here are in harmony with previous observations in calcium metabolism work with cows. There is no indication, however, that the different treatments to which the hays had been subjected materially influenced the availability of the calcium. The calcium in the immediately dried hay appears to have been a little more readily assimilated, but the difference is very slight.

TABLE 3.—Calcium balance and milk production of three cows when fed alfalfa hay which had been dried and cured in different ways

COW 1—PERIOD 1—HAY 1 (DRIED IMMEDIATELY)

Week No.	CaO in feces	CaO in milk	CaO in urine	Total CaO excreted	Total CaO intake	CaO balance	Weekly milk production
	Grams	Grams	Grams	Grams	Grams		Pounds
1.....	708.29	251.57	7.59	967.45	895.28	-72.17	432.8
2.....	699.93	246.49	15.61	962.03	895.28	-66.75	448.7
3.....	733.28	233.06	10.49	977.73	895.28	-82.45	422.4

COW 1—PERIOD 2—HAY 2 (PARTLY SUN CURED)

4.....	677.06	251.39	9.96	938.41	803.71	-134.70	436.0
5.....	672.95	254.82	9.95	937.72	803.71	-134.01	412.7
6.....	669.17	250.43	6.50	926.10	803.71	-122.39	427.6

TABLE 3.—*Calcium balance and milk production of three cows when fed alfalfa hay which had been dried and cured in different ways—Continued*

COW 1—PERIOD 3—HAY 3 (SUN CURED)							
Week No.	CaO in feces	CaO in milk	CaO in urine	Total CaO excreted	Total CaO intake	CaO balance	Weekly milk production
	Grams	Grams	Grams	Grams	Grams		Pounds
7.....	623.51	233.57	16.04	873.12	753.50	-119.62	414.9
8.....	609.93	221.03	11.66	902.62	753.50	-149.13	405.7
9.....	636.63	193.41	15.44	845.48	753.50	-91.98	377.0
COW 2—PERIOD 1—HAY 1 (DRIED IMMEDIATELY)							
1.....	689.56	248.96	9.83	948.35	890.59	-57.76	340.6
2.....	661.23	248.01	10.70	919.94	890.59	-29.35	313.3
3.....	614.63	246.74	7.82	869.19	890.59	+21.40	323.5
COW 2—PERIOD 2—HAY 2 (PARTLY SUN CURED)							
4.....	628.21	243.83	8.49	880.53	738.43	-82.10	317.8
5.....	593.57	247.49	7.63	848.69	798.43	-50.26	313.3
6.....	611.58	231.43	9.82	852.83	798.43	-54.40	318.6
COW 2—PERIOD 3—HAY 3 (SUN CURED)							
7.....	637.53	231.40	10.71	879.64	748.22	-131.42	301.6
8.....	583.55	222.90	18.07	824.52	748.22	-76.30	204.0
9.....	400.78	149.28	6.65	556.71	748.22	+191.51	106.9
COW 3—PERIOD 1—HAY 1 (DRIED IMMEDIATELY)							
1.....	635.22	271.46	10.98	917.66	895.28	-22.38	427.1
2.....	634.68	249.07	8.55	892.30	895.28	+2.98	428.6
3.....	660.05	206.67	11.79	938.51	895.28	-43.23	435.1
COW 3—PERIOD 2—HAY 2 (PARTLY SUN CURED)							
4.....	662.44	239.28	5.47	907.19	803.71	-103.48	428.5
5.....	623.66	204.92	7.50	926.17	803.71	-122.46	430.2
6.....	553.28	271.31	7.75	832.34	803.71	-28.63	436.2
COW 3—PERIOD 3—HAY 3 (SUN CURED)							
7.....	601.28	263.70	5.41	870.39	753.50	-116.89	420.9
8.....	612.06	265.59	5.89	883.54	753.50	-130.04	414.9
9.....	582.63	229.05	7.35	819.03	753.50	-65.53	388.1

* Cow off feed.

SUMMARY

Second-cutting alfalfa hays, (1) dried immediately in an artificial drier (Koon); (2) partly dried in the field and then dried in the artificial drier, and (3) completely dried in the field, were studied for the availability of their dry matter, protein, and calcium.

The availability of the dry matter, protein, and calcium was much alike in all three hays, as measured by metabolism experiments with heavy-milking cows.

Apparently the high temperature of 480° to 535° C. for 40 seconds, to which the green legume plants were exposed in the immediate-drying process, did not reduce the availability of the nutrients studied.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 45

WASHINGTON, D. C., NOVEMBER 1, 1932

No. 9

A CYTOLOGICAL STUDY OF HETEROTHALLISM IN *PUCCINIA CORONATA*¹

By RUTH F. ALLEN²

*Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry,
United States Department of Agriculture, and Associate in Agronomy, California
Agricultural Experiment Station*

INTRODUCTION

Since the announcement by Craigie in 1927 of heterothallism in the rusts (11)³ and his subsequent investigations confirming and extending his observations (12, 13), work has gone steadily forward on this subject.

Waterhouse (26), Stakman, Levine, and Cotter (25), and Newton, Johnson, and Brown (22) hybridized physiologic forms of *Puccinia graminis* Pers. and found in the progeny physiologic forms of the rust new to science.

Hanna (16) published a preliminary account of cytological studies of *Puccinia graminis* showing that the isolated monosporidial infection remains haploid and produces haploid aecia. When spermatia (pyniospores) of opposite sex are transferred to it, the spermatia germinate, and soon after this the sporophyte generation originates in the aecium in the manner described by Christman (8, 9).

The writer (1), in a paper covering the same ground, found that in the infection of *Puccinia graminis* to which spermatia have been transferred the sporophyte generation begins earlier. Mycelial cells containing from one to five nuclei are found at the spermogonia, at various points in the mycelium, and in the young aecium before the sporogenous layer is differentiated.

Andrus (3), in a cytological study of *Uromyces appendiculatus* (Pers.) Fries on beans and *Uromyces vignae* Barclay on cowpeas, found in the aecial primordia certain uninucleate 2-legged cells, which are considered equivalent to eggs. Each is joined to a "foot cell," and from each a hypha, trichogynous in nature, grows upward, branching as it grows, to the upper epidermis (and to a lesser degree to the lower), where the tips project into stomata or between epidermal cells. Spermatia on the surface of the leaf enter the ruptured tips of

¹ Received for publication Mar. 2, 1932; issued November, 1932. Cooperative Investigations, Agricultural Experiment Station of the University of California and Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture.

² Acknowledgments are made to W. F. Fraser, of the University of Saskatchewan, Saskatoon, Canada; M. Newton, of the Dominion Rust Research Laboratory, Winnipeg, Canada; L. D. Leach, of the California Agricultural Experiment Station; and H. B. Humphrey and H. C. Murphy, of the Division of Cereal Crops and Diseases, for spores of *Puccinia coronata*; to H. C. Murphy and L. D. Leach for seed of *Rhynchospora cathartica*; and to the members of the divisions of agronomy and genetics of the University of California for courtesies extended during the work.

³ Reference is made by number (italic) to Literature Cited, p. 540.

these trichogynous hyphae, and their nuclei travel through them to the egg, which then, either directly or after further proliferation, gives rise to spores.

In a similar study of *Puccinia triticina* Eriks. the writer (2) found that the isolated infection of monosporidial origin remains haploid. Spermatogonia form in about equal numbers at both surfaces of the leaf. While spermatogonia are developing, hyphae grow into stomatal apertures at the lower surface of the leaf or between epidermal cells at either leaf surface. Aecial primordia form near the stomatal hyphae. When spermatia from another and different infection are applied to the surface of an infected area, spermatial nuclei may enter at any exposed hypha tip, initiating there the sporophyte generation, which then spreads quickly from the point or points of origin and soon permeates the aecia. The spread may be by growth, perhaps also by "diploidization," as described by Buller (7). Multinucleate cells are common during this development, both in the mycelium and in the aecia.

The theories of Blackman (5, 6) and Christman (8, 9) as to the origin in ontogeny of the sporophyte generation in rusts are somewhat at variance with each other. Recent advances in our knowledge of heterothallism and the function of spermatia in this group have brought both of the older theories under question. It was in the hope of obtaining further light on the subject that a cytological study of *Puccinia coronata* Cda. was undertaken.

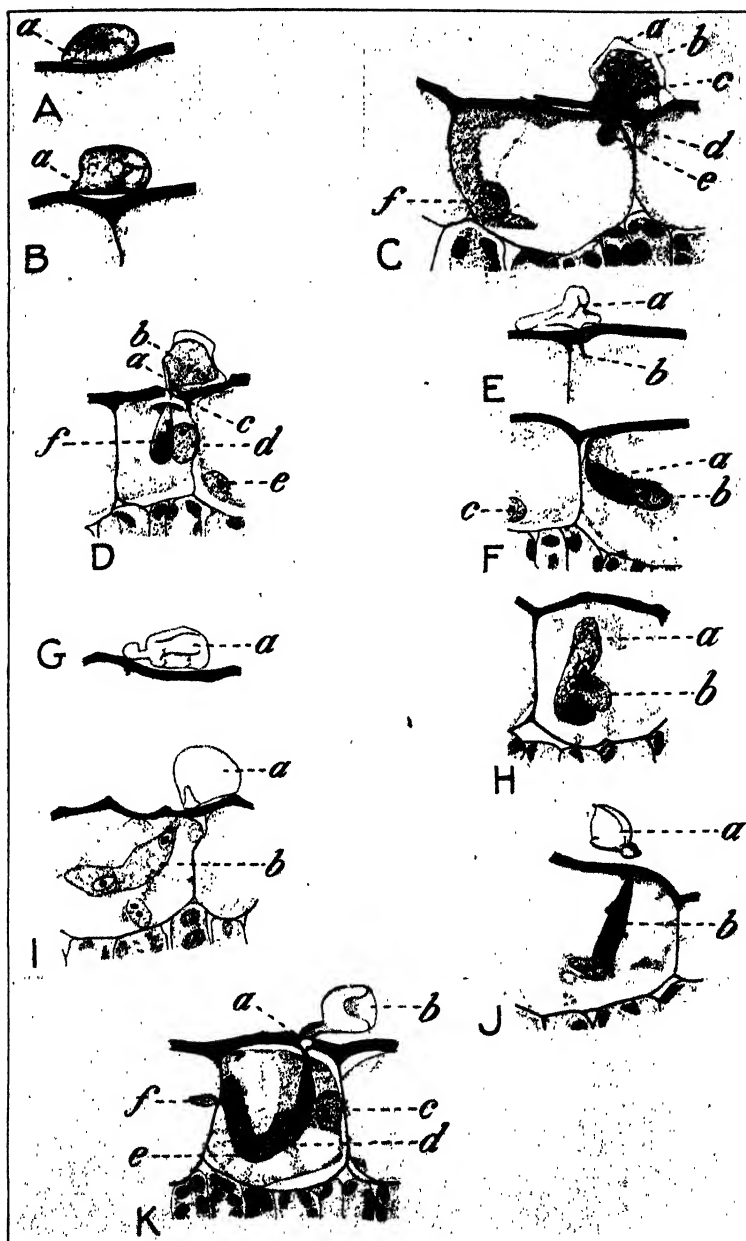
MATERIALS AND METHODS

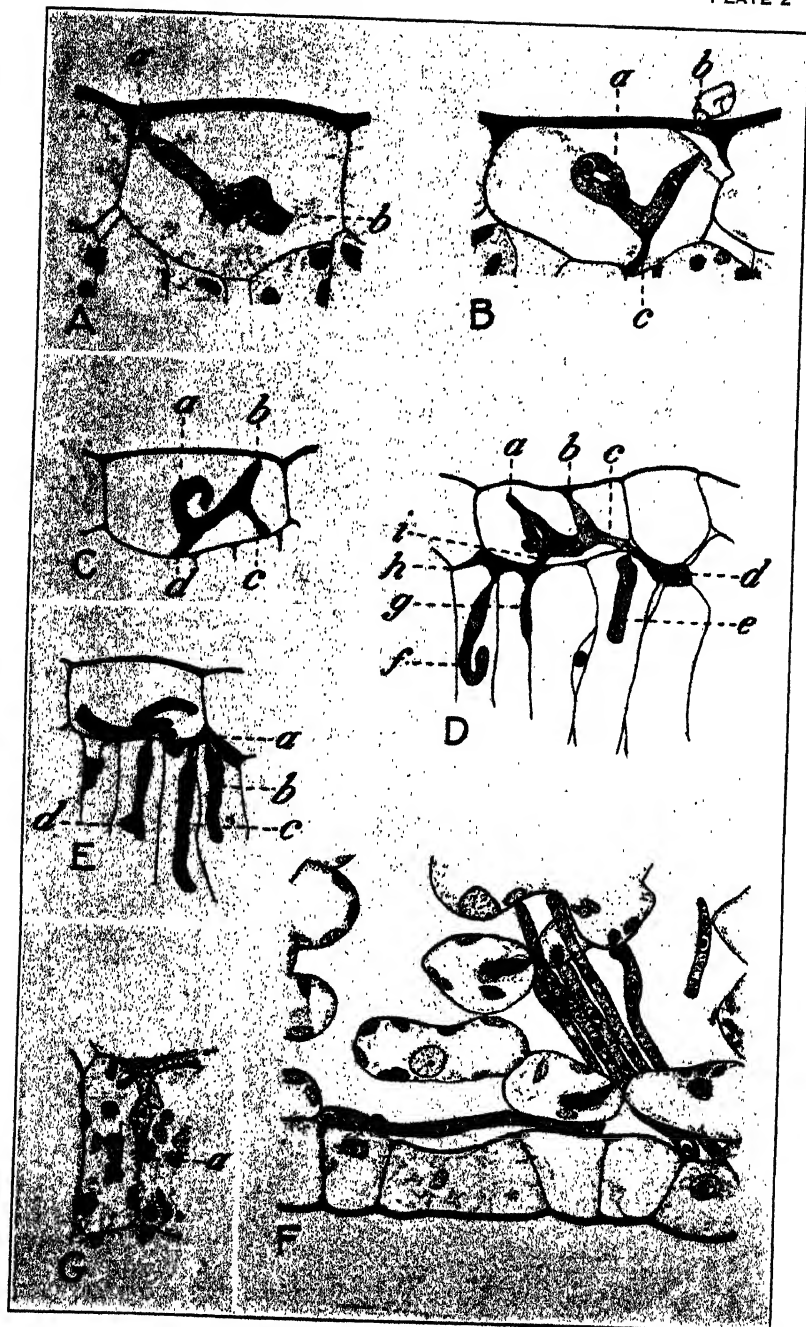
Spores of *Puccinia coronata* were stored, until time for use, in loose-mesh cloth bags on the ground in a partly shaded spot, or in the head house (potting house). Buckthorn plants (*Rhamnus cathartica* L.) were grown in the greenhouse and used when they were from 4 to 10 inches high.

In inoculating, watch crystals were filled with mud. Bits of rusted oat leaves were soaked in rain water several hours and sprayed with an atomizer, then pressed into the mud with the spores exposed, and sprayed again. A bit of wet sphagnum was wrapped around the base of each buckthorn seedling. Tall glass tumblers with the bottoms removed and the sides lined with wet paper were placed over the seedlings, and the mud-filled crystals were used as lids. A layer of wet paper was folded down over the top of each tumbler and held in place with a rubber band. The whole was placed under a greenhouse bench for 48 hours, then uncovered and placed on the bench. The plants were kept covered with tarlatan cages to exclude insects.

EXPLANATORY LEGEND FOR PLATE 1

- A.—Uninucleate sporidium of *Puccinia coronata* on the surface of a *Rhamnus* leaf, pushing out a beak at a.
- B.—Uninucleate sporidium, with narrower beak (a).
- C.—Beginning of infection: a, Sporidial wall; b and c, nuclei in cytoplasm; d, pierced epidermal wall; e, entering cytoplasm; f, host nucleus.
- D.—Stage of entrance: a and b, Nuclei in the sporidium; c, pore in the wall at the point of entrance; f, club-shaped mass of fungus plasma in cell; d and e, host nuclei.
- E.—One-day infection: a, Collapsed sporidial wall; b, entrance tube.
- F.—One-day infection: a, Binucleate fungus cell in epidermal cell; b and c, host nuclei.
- G.—One-day infection: a, Collapsed sporidial wall.
- H.—One-day infection: a, Binucleate fungus cell in epidermal cell; b, host nucleus.
- I.—Two-day infection: a, Empty sporidial wall; b, binucleate fungus cell.
- J.—Two-day infection: a, Sporidial wall; b, trinucleate fungus cell inside.
- K.—One-day infection: a, Entrance pore; b, sporidial wall; d and e, septa of 3-cell primary hypha in epidermal cell; f, branch from primary hypha; c, host nucleus. (All $\times 1,130$.)





Material was fixed daily for the first three weeks and at longer intervals from then on until the death of the fungus. Several fixing solutions were used. Flemming's weak was the most trustworthy, but Flemming's medium was equally good with older material. After remaining 36 to 48 hours in the fixing fluid, the material was washed, dehydrated, and embedded in 50° paraffin. The principal stains used were safranin and methylene blue. These are excellent when the slides are fresh. Prolonged exposure to light, however, fades the blue stain.

EXPERIMENTAL DATA

ENTRY OF THE RUST

Twenty-four hours after the *Rhamnus* plant was placed in a damp chamber, with the rusted oat leaves above it, some of the teliospores germinated and produced and discharged the sporidia, or basidiospores. The sporidia fell on the leaves, where some of them germinated and entered the host, forming hyphae of several cells within the epidermal cells. The steps in this last process have been studied.

The sporidium is a small, thin-walled, short-lived spore containing a single nucleus and cytoplasm with scant food supply. In the presence of moisture it germinates promptly, forming a short, broad process at one end (pl. 1, A, *a*)⁴ or a narrower beak (pl. 1, B, *a*), or, more rarely, a short germ tube almost equaling in length the diameter of the spore. The tip of this beak becomes closely applied to the epidermal wall, and entry is effected at that point. By the time entry has begun, the sporidial nucleus has usually divided into two. (Pl. 1, C, *b*, *c*; D, *a*, *b*.)

In the uredinal generation the spore produces a long germ tube, often many times the diameter of the spore in length, which grows to the nearest stoma and enters through the stomatal aperture. That method of entry is not feasible here. No stomata have been observed on the upper surface of *Rhamnus* leaves, where at least 99 per cent of the entries take place. The fungus enters directly through the thick outer epidermal wall. The means by which this wall is perforated, whether chemical or mechanical or a combination of the two, has not been determined. The opening in the wall is large enough to be visible under the microscope. (Pl. 1, C, *d*; D, *c*; E, *b*; K, *a*; pl. 2, A, *a*; B, *b*.)

The entering cytoplasm forms a rounded mass within the epidermal cell (pl. 1, C, *e*), which soon expands into a club-shaped body extending toward the inner face of the cell. (Pl. 1, D, *f*.) This curves so that the distal end lies free in the center of the epidermal cell. (Pl. 1, F, *a*.) In this case the empty collapsed sporidial wall (pl. 1, E, *a*)

⁴ For the sake of uniformity and clearness, the drawings are oriented in the plates as the tissues are in the leaf, i. e., with the tissues nearest the upper surface of the leaf uppermost in the drawing.

EXPLANATORY LEGEND FOR PLATE 2

- A.—Two-day infection: *a*, Entrance pore; *b*, 5-cell primary hypha. $\times 1,130$.
 B.—Two-day infection: *b*, Entrance pore in sporidial wall; *a*, 4-cell primary hypha with one branch (c). $\times 1,130$.
 C.—Three-day infection (outlines only): *a* and *b*, 3-cell primary hypha with two branches (*c*, *d*). $\times 1,130$.
 D.—Three-day infection (outlines only): *b*, Primary hypha with branches (*a*, *c*, *f*) giving rise to sub-epidermal hyphae (*d*, *h*), a hypha (*g*) growing down palisade cells, and haustoria (*e*, *j*). $\times 1,020$.
 E.—Three-day infection (outlines only): *a*, Subepidermal hyphae; *b*, 2-cell haustorium; *c*, 3-cell haustorium; *d*, 1-cell haustorium. $\times 1,020$.
 F.—Four-day infection from an entrance through the lower epidermis of leaf. Hyphae growing freely in air spaces. $\times 1,020$.
 G.—Detail of 4-day infection: *a*, Hypha forcing a passage between palisade cells. $\times 1,020$.

and the entrance tube (pl. 1, E, *b*) were found in the next section. Similar examples are shown in Plate 1, G, H, and I.

The transferred protoplasm of the sporidium is now an elongated, often sausage-shaped mass in the interior of the host cell. It is still unicellular and binucleate. As entry progresses, the delicate sporidial wall outside collapses irregularly. The host nucleus, normally lying against the inner wall of the epidermal cell (pl. 1, C, *f*; D, *e*; F, *c*), is now commonly in contact with the fungus (pl. 1, D, *d*; F, *b*; H, *b*; K, *c*).

A septum may now divide the young primary hypha into two cells, or there may be another nuclear division before septation. In Plate 1, J, the cell (*b*) contains three nuclei. Plate 1, K, shows a 3-cell hypha formed from such a trinucleate cell. The fungus is overstained, so that the details of the contents are vague, but the septa (pl. 1, K, *d*, *e*) are unmistakable.

The primary hypha usually develops further by apical growth, becoming 4-cell, 5-cell, or even 6-cell, and irregularly bent or coiled within the host cell. In Plate 2, A, the primary hypha has five cells, and from the truncated appearance of the tip (*b*) at least one other cell was lost in sectioning.

DEVELOPMENT OF MYCELIUM

On the second day, the primary hypha branches, and the subepidermal region may be invaded. With few exceptions each cell of the primary hypha branches in turn, beginning with the oldest. In Plate 2, B, only the first cell has branched (*c*). In Plate 2, C (drawn in outline only), the first and second cells have branched (*c*, *d*). In Plate 2, D (outlines only), branches from the first, second, and third cells have formed at *c*, *i*, and *a*, respectively. Usually these branches of the primary hypha grow directly toward the inner wall of the epidermal cell and push through into the subepidermal area. More rarely, a branch may enter the next epidermal cell (pl. 1, K, *f*) or even emerge upon the outer surface of the leaf.

In entering the subepidermal region, the fungus encounters mechanical obstruction. The palisade layer of the *Rhamnus* leaf is very compact and is closely applied to the epidermis. Intercellular spaces are few and small. Plate 2, D, already referred to, shows (outlines only) a central section through a 3-day infection. Two branches of the primary hypha (*c*, *i*) have passed through the inner wall of the epidermal cell and formed the intercellular hyphae (*d*, *h*) by separating the host cells and growing between them.

Whether this splitting of host-cell walls is achieved by mechanical or chemical means is not clear, but it is probably the former. There

EXPLANATORY LEGEND FOR PLATE 3

A.—Median section of 4-day infection: *a* to *f*, Subepidermal mycelium; *c*, primary hypha; *b*, *d*, *e*, hyphae growing down, the last reaching intercellular spaces below (*g*, *h*). $\times 640$.

B.—Detail of 4-day infection: *a*, Flat view of wedge-shaped hypha forcing passage between palisade cells. $\times 1,020$.

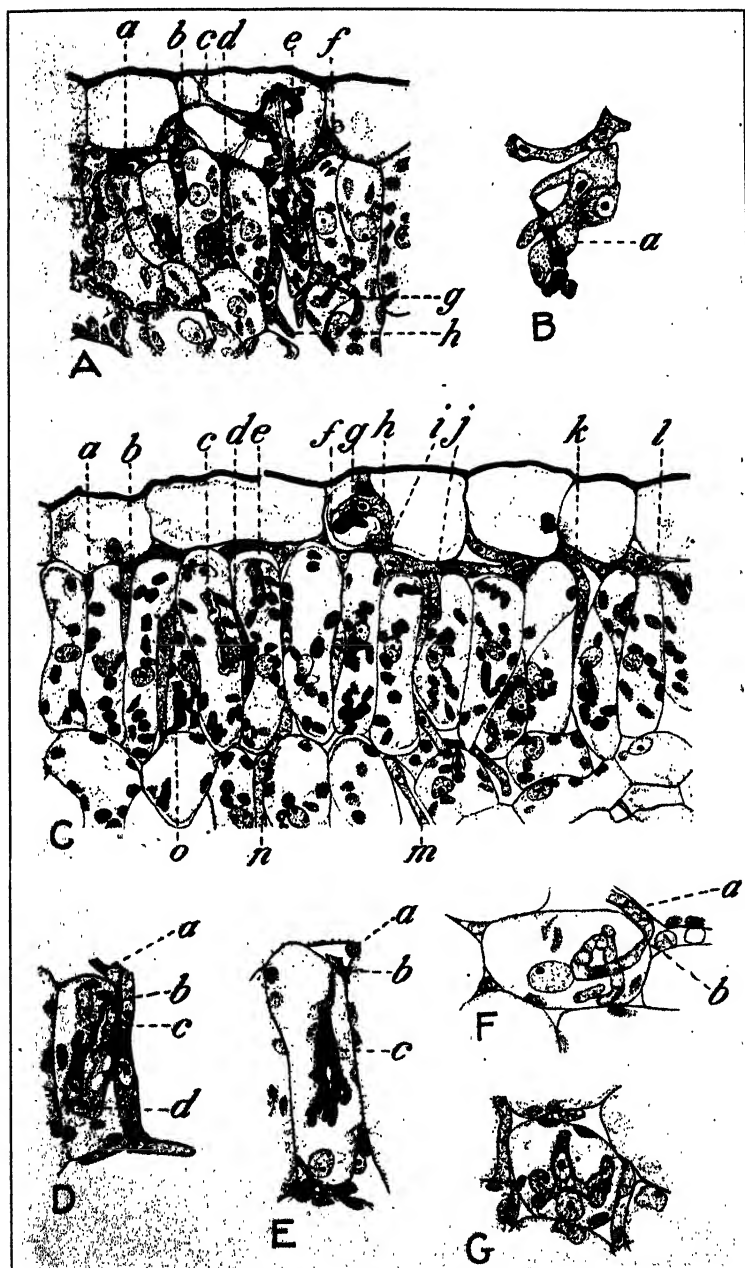
C.—Central section of 6-day infection: *a* to *i*, Subepidermal mycelium; *f*, *g*, *h*, and *i*, remnant of primary hypha; *a*, *b*, *d*, *j*, and *k*, hyphae between palisade cells; *m* and *n*, hyphae in air spaces below palisade; *c*, *e*, and *o*, haustoria. $\times 730$.

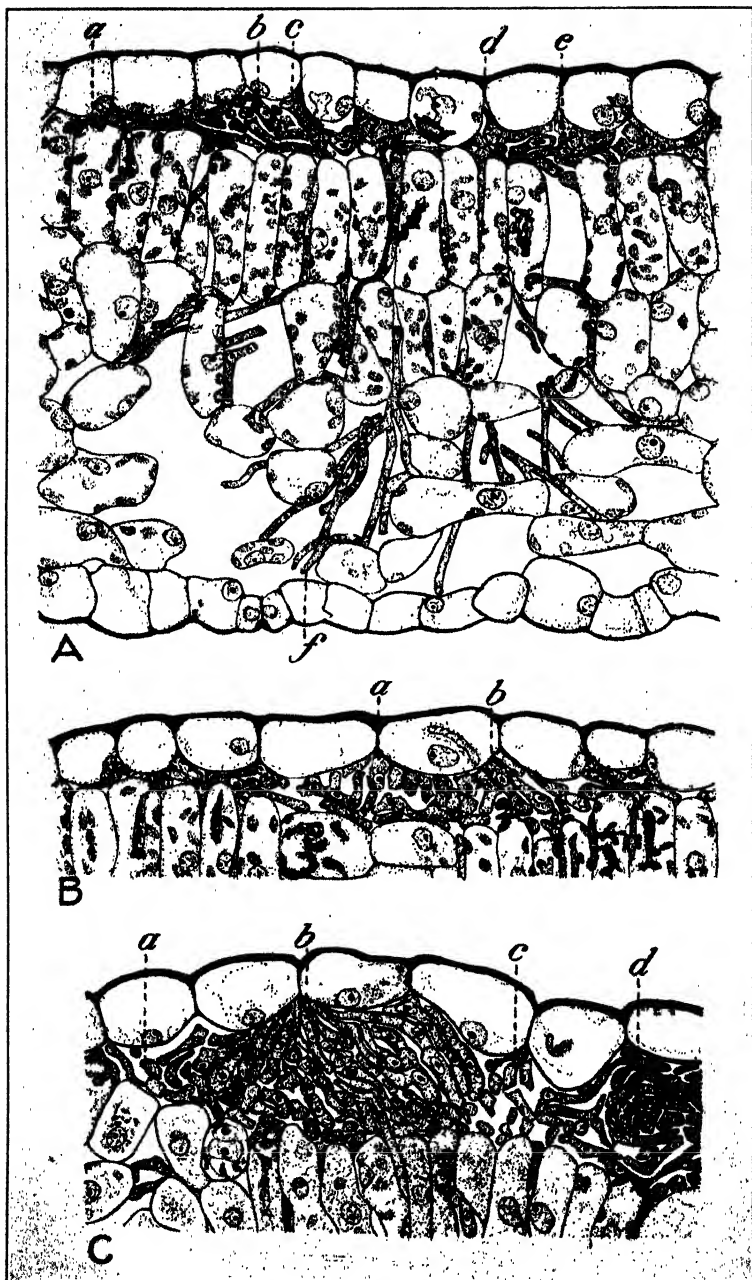
D.—Detail from 4-day infection: *a*, Haustorium initial; *b*, entrance to host cell; *c* to *d*, branchless bent haustorium. $\times 1,020$.

E.—Detail from 7-day infection: *a*, Haustorium initial; *b*, entrance; *c*, dichotomously forked haustorium. $\times 1,020$.

F.—Detail from 4-day infection: Cytoplasm visibly continuous at *b*, between haustorium initial (*a*) and haustorium. $\times 1,020$.

G.—Detail from 4-day infection showing dichotomously forked haustorium in mesophyll cell. $\times 1,020$.





is no evidence of swelling and softening of the middle lamellae of the walls such as one would expect if they were being dissolved. Moreover, the advancing tip of the hypha often forms a flattened, sharp-edged wedge, well fitted for forcing a passageway. The edge view of such a hypha is seen in Plate 2, D, at *h*; Plate 2, G, at *a*; and Plate 3, C, at *a*, *b*, and *k*. The appearance in flat view is shown in Plate 3, B, at *a*.

The initial intercellular growth of the rust is between the epidermal and the palisade layers. In order to reach the more open spongy mesophyll of the leaf, the fungus must either grow down through the palisade cells or force a passage between them. In Plate 2, D, both methods are being essayed. At *g* is a hypha making its way down between two palisade cells. At *e* and *f* are intracellular growths intermediate in character between haustoria and hyphae. These may be 1, 2, or 3 cells in length. (Pl. 2, D, *e, f*; E, *b, c, d*.) Although they are like hyphae in appearance, they are probably to be regarded as haustoria. No case of successful passage through a palisade cell into the air spaces beneath has been noted.

Rarely, a sporidium enters through the lower epidermis of the leaf. In this case the mycelium reaches spongy mesophyll at once (pl. 2, F) and spreads freely through the air spaces.

A slightly later stage of development is shown in Plate 3, A. From the primary hypha (*c*), now more or less disintegrated, subepidermal hyphae have formed and spread from *a* to *f*. Branches from these are working their way down between palisade cells at *b*, *d*, and *e*, and at two points (*g*, *h*) have reached the freedom of larger air spaces.

The tendency to spread just beneath the epidermis persists as development continues. Plate 3, C, shows a central section through a 6-day infection. This material was fixed early in the season when the fungus grew slowly. Later in the spring a 6-day infection would be forming spermatogonia. The primary hypha (pl. 3, C, *h*) is still recognizable. At *f* and *i* are points of entry into subepidermal spaces. There is now a continuous mat of subepidermal hyphae, from *a* to *l*, still only one cell thick for the most part, but in places thicker (*j*, *k*, *l*). There are several hyphae working down between palisade cells (*a*, *b*, *d*, *j*, *k*) and a few deeper in the leaf (*m*, *n*). The cells of the mycelium are regularly uninucleate.

Not all the haustoria formed in palisade cells are hyphalike. Plate 3, D, shows one of quite a different type. The haustorium initial (*a*) effected an entry into the host cell at *b*, and the haustorium has grown down to *d* and doubled back to *c*. It is a single unbranched cell, with the uneven diameter and irregular outline common to haustoria. Some of the haustoria fork dichotomously at the inner end. Cases of repeated dichotomy are figured in Plate 3, E and G. A distinguishing trait of gametophytic haustoria of crown rust is the presence of cytoplasm (but no nucleus) in the haustorium initial after the haustorium is fully formed. (Pl. 3, D, *a*; E, *a*; F, *a*.) Another difference is the comparatively large pore formed in the host cell wall at the point of entry. (Pl. 3, D, *b*; E, *b*; F, *b*.) The cytoplasm of haustorium and haustorium initial may be visibly continuous.

EXPLANATORY LEGEND FOR PLATE 4

- A.—Transverse section of leaf through center of 6-day infection: *a* and *e*, Subepidermal stroma; *b* and *e*, beginnings of spermatogonia; *c* and *d*, receptive hyphae; *f*, mycelium spreading through air spaces below.
 B.—Slightly later stage (6-day infection) with spermatogonia started at *a* and *b*.
 C.—Six-day infection: Young spermatogonia organized at *b* and *d*; looser broken hyphae between, at *a* and *c*.
 (All $\times 640$.)

Several cases have been found in which two or even three contiguous epidermal cells contained primary hyphae. The mycelia developing from these became intimately interwoven. While, on the basis of heterothallism, some of these double infections may be both (+) or both (-), it is unlikely that all of them are. In the cases of treble infection, the chances that all three are of the same sex are still further reduced. Yet a careful survey of these multiple infections shows regularly uninucleate cells. There is no apparent attraction between (+) and (-) elements at this stage.

SPERMOGONIA AND RECEPTIVE HYPHAE

The mycelium grows rapidly, permeating the intercellular spaces of the spongy mesophyll (pl. 4, A, *f*) and pushing out farther between the epidermis and the palisade (pl. 4, A, *a*, *e*). The dominant growth is still just beneath the epidermis. The subepidermal mat, or stroma, has spread marginally and thickened at the center. In spots it is now four or five cells thick (pl. 4, A, *b*, *e*), and although there is no recognizable organization of the hyphae here, it is at such points as these that the spermogonia originate. Throughout the area of the mat the epidermis has been separated from the palisade and is being lifted by the growing fungus beneath it.

At this time several centers of development appear in the stroma, in each of which a small circular patch of cells pushes up a dense growth of short, upright hyphae, slanted somewhat toward the center of the patch. Plate 4, B, *a* to *b*, represents such a center. This is the beginning of a spermogonium.

A somewhat later stage in the development of the spermogonium (also from a 6-day infection) is shown in Plate 4, C. The upright hyphae have now converged upon the common point *b*. At *d* is the edge of the next spermogonium. The growth of the fungus between spermogonia has not kept pace with this, so there are open spaces between the hyphae at *a* and *c*.

Soon after this the different kinds of cells within the spermogonium become differentiated. In Plate 5, A, the slender, tapering hyphae just beneath the epidermis and nearly parallel to it at *a* and *d* will become the paraphyses. The heavy central upright hyphae at *b*, with coarsely alveolar cytoplasm and blunt tips pressed against the epidermis, are the buffer cells, whose main function is to lift the epidermis and withstand its pressure. Growing in between the buffer cells at the base are slender pointed cells at *c* and *e*. These are the first of the spermatophores.

The epidermis above the spermogonium is strained, and its cells are somewhat flattened. The lifting of the epidermis at the spermogonia also raises it, to a lesser extent, between them. The few hyphae of the subepidermal mat between the spermogonia (pl. 5, A, *f*) are often displaced and broken by the lifting of the epidermis.

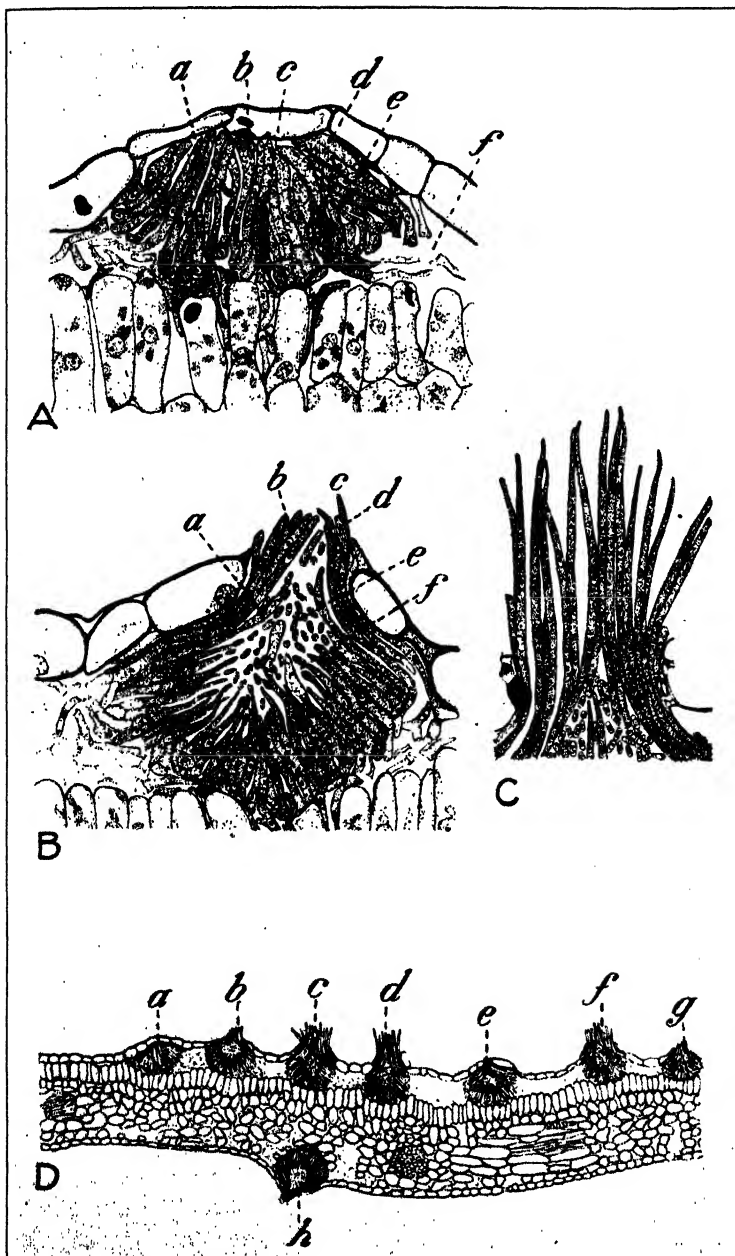
EXPLANATORY LEGEND FOR PLATE 5

A.—Spermogonium from 6-day infection: *a* and *d*, Young paraphyses; *b*, buffer cells; *c* and *e*, spermatophores; *f*, broken, dying hyphae. $\times 640$.

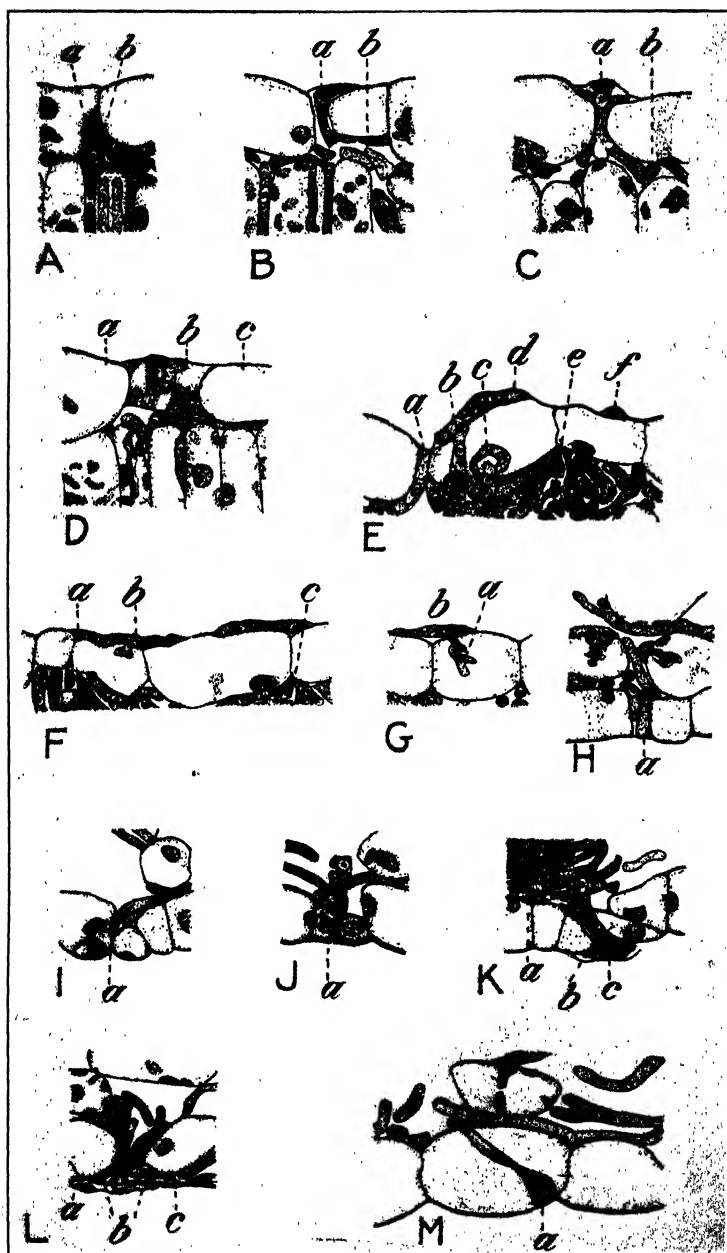
B.—Six-day infection; spermogonium just opening: *b* and *c*, Paraphyses; *a* and *f*, buffer cells; *e*, spermatia. $\times 640$.

C.—Seven-day infection: Tuft of paraphyses extruded from ostiole of spermogonium. $\times 640$.

D.—Semiagrammatic drawing of median section of 7-day infection: *a* to *g*, Subepidermal welt between palisade and epidermis, bearing spermogonia at *a*, *b*, *c*, *d*, *e*, *f*, and *g*; smaller stroma and spermogonium (*h*) at lower epidermis. $\times 115$.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE



The spermatophores (pl. 5, B) now increase rapidly in numbers. Cells along the base and sides of the spermogonium branch and grow in toward the center. The interpolation of new spermatophores among the old enlarges the spermogonium and rounds it out at base and sides. As it arches outward a central cavity forms (pl. 5, B, *e*) and into this the spermatophores set free the spermatia. The buffer cells, which earlier formed the central columns, braced against the epidermis (pl. 5, A, *b*), are now separated from each other by the spermatophores (pl. 5, B, *a, f*) and can be seen here and there jutting into the central cavity. The paraphyses in the meantime have converged and turned outward (pl. 5, B, *b, c*), breaking through the epidermis and forming the ostiole. These paraphyses (pl. 5, C) elongate into a brush of straight tapering hyphae. So far as noted, the extruded part of a paraphysis is nonseptate and contains one nucleus or, more commonly, two nuclei.

Some idea of the appearance of the infection as a whole at this stage may be obtained from the diagram of a central section through a well-developed 6-day infection in Plate 5, D. There are spermogonia at different stages of development on the stroma at *a, b, c, d, e, f*, and *g*. The areas beneath the epidermis between spermogonia appear almost empty. The few hyphae of the subepidermal stroma between the spermogonia are broken, and often dead and nearly transparent. A little later, however, this space is filled in by a secondary growth of hyphae.

Usually a similar but smaller stroma, which bears a few spermogonia, forms at the lower surface of the leaf. Plate 5, D, *h*, shows the beginning of such a stroma. A count of 100 spermogonia in 6-day infections shows that 73 open upon the upper surface of the leaf and 27 on the lower. A similar count in 10-day infections gives 77 on the upper surface and 23 on the lower.

Spermogonia and receptive hyphae usually develop at the same time. In Plate 4, A, already referred to, spermogonia are forming at *b* and *e*, and receptive hyphae are pushing out between the cells of the upper epidermis at *c* and *d*. In Plate 6, A, two hyphae (*a, b*) have split the epidermal walls apart for two-thirds of the distance to the leaf surface. In Plate 6, E, the hypha (*a*) has reached the surface, and its tip is flattened against the cuticle of the leaf.

The first receptive hyphae antedate the accia by several days and may even antedate the spermogonia, as in Plate 6, B and C. In

EXPLANATORY LEGEND FOR PLATE 6

- A.—Detail of 6-day infection: *a* and *b*, Two hyphae pushing up between cells of the upper epidermis.
- B.—Six-day infection: *a*, Hypha grown out between epidermal cells and on a short distance beneath the cuticle; *b*, subepidermal mycelium.
- C.—Six-day infection: *a*, Hypha grown out between cells of the upper epidermis, with three divergent branches; *b*, subepidermal mycelium.
- D.—Six-day infection: *a* to *c*, Detail of upper epidermis; *b*, hyphae growing toward surface of leaf.
- E.—Detail of 6-day infection: *a*, Inter-cellular receptive hypha; *c*, haustorium; *e*, young spermogonium from which an intracellular hypha (*b*) grew up and gave rise to the subcuticular hypha *d, f*, cross section of subcuticular hypha.
- F.—Six-day infection: *a*, Subcuticular growth from which a haustorium is forming at *b*; *c*, young spermogonium beneath the epidermis.
- G.—Detail of upper epidermis in 6-day infection: *b*, Subcuticular hypha, from which a haustorium has formed at *a*.
- H.—Detail of 7-day infection: *a*, Hyphae between cells of the lower epidermis.
- I.—Detail of 7-day infection: *a*, Hypha wedged into the inner angle of a shut stoma.
- J.—Detail of 7-day infection: *a*, Hypha wedged into the inner angle of a shut stoma.
- K.—Detail of 7-day infection: *a*, Paraphyses; *b*, membrane; *c*, broad-top emergent hypha in stoma (cut obliquely) adjoining a half-grown spermogonium.
- L.—Detail of 7-day infection: *a* and *c*, Dead cells of lower epidermis; *b*, hyphae crowding down against lower epidermis.
- M.—Eight-day infection: *a*, Intracellular receptive hypha at the lower epidermis. (All $\times 640$.)

Plate 6, B, a hypha (*a*) has grown up between the epidermal cells and after reaching the outer epidermal wall has grown farther, parallel to the leaf surface, underneath the cuticle. These hyphae seem to be unable either to pierce the cuticle mechanically or to dissolve it chemically but are able to effect a separation between the cuticle and the inner layers of the epidermal wall and to grow for considerable distances beneath the cuticle. Sometimes a hypha emerging upon the surface gives rise to a single subcuticular hypha, sometimes to several. In Plate 6, C, the hypha at *a* is forming three young branches, which have started off in different directions under the cuticle.

The infection from which Plate 6, B and C, were drawn is small. It is in a slightly earlier stage of development than the one shown in Plate 4, A. The subepidermal mat of hyphae is still thin (pl. 6, B, *b*; C, *b*) and without signs of spermatogonia. The mycelium has not yet reached the lower epidermis of the leaf, and it will be several days before the first aecium is initiated. There is no possibility here that these emergent hyphae originated in an aecial primordium.

Emergent hyphae usually grow up between epidermal cells, but there is some evidence that they can also push up through the host cell. In Plate 6, D, is a case open to two interpretations. It may be that the hyphae at *b* are seen in flat view as they push up between epidermal walls lying in the plane of the section. It is also possible that they are growing up intracellularly. Certainly the host cell (*b*) has lost turgidity, for the side walls of the adjoining epidermal cells (*a*, *c*) are bulging into it.

In Plate 6, E, the evidence is clearer. The hypha (*b*) grew straight up through the epidermal cell and then grew on as a subcuticular hypha (*d*). The habit of growth of the receptive hypha (*b*) is in marked contrast to that of the haustorium (*c*) alongside it. Subcuticular hyphae grow in a cramped space and are more or less flattened by pressure, as may be seen from the cross section of such a hypha. (Pl. 6, E, *f*.) In this instance receptive hyphae and spermatogonia are closely associated, for there is a young spermatogonium below the epidermis at *e*.

These subcuticular hyphae, like the mycelium producing them, are septate and haploid. Also, like the mycelium below, they have not lost the ability to produce haustoria. When a hypha has grown subcuticularly for some distance, its growing tip is remote from the base of supplies. At *b*, in Plate 6, F, a young haustorium is forming from an outer hypha and in Plate 6, G, at *a*, is a full-grown haustorium, which entered from the outer face of the epidermal cell. So far as appearances go, these subcuticular hyphae differ from vegetative hyphae only in their location.

When the spermatogonia are young and the subepidermal mycelium is vigorous, emergent hyphae with subcuticular extensions are formed in abundance. A little later, as the spermatogonia mature, lifting the upper epidermis as they grow, the subepidermal hyphae between spermatogonia become broken and almost disappear. If the connections to the surface hyphae are broken they, too, shrivel and disappear. This subepidermal space is soon filled in by a secondary growth of mycelium, however, and new emergent hyphae form similar to the first lot.

Meanwhile, the vegetative mycelium of the young growing infection is spreading in all directions and soon reaches the lower epidermis of

the host leaf. Here a new series of receptive hyphae is formed, some pushing into stomatal apertures (pl. 6, I, J, K), others squeezing between epidermal cells (pl. 6, H), and still others growing through an epidermal cell (pl. 6, M).

Plate 6, H, drawn from a 7-day infection, shows a bit of the lower epidermis between two cells of which, at *a*, hyphae have grown. One has reached the lower surface of the leaf but is still inclosed by the cuticle. These receptive hyphae and the mycelium above them are haploid.

Hyphae are to be found in stomatal apertures but are not nearly so abundant as in *Puccinia triticina*. In Plate 6, I and J, the stoma is closed, but the hyphae above it are definitely aimed at the small crack between the guard cells. In Plate 6, K, in which the stoma is cut obliquely, the hypha has been thrust through the stomatal opening (pl. 6, K, *c*) and has broadened out at the tip. A sort of thin film or membrane (pl. 6, K, *b*) covers the exposed tip of the hypha. This has frequently been observed in stomatal hyphae and may represent a substance secreted by the hypha.

This emergent hypha (pl. 6, K, *c*) has formed next to a half-grown spermogonium, and the hypha itself has grown out of the group of young paraphyses (pl. 6, K, *a*) of the spermogonium. This is of fairly frequent occurrence and would seem to indicate that there is no potential difference between a paraphysis and a receptive hypha. So far as can be determined, the same hypha can develop into either structure.

In Plate 6, L, a minor injury killed two or three epidermal cells (pl. 6, L, *a*, *c*), and no less than five hyphae (pl. 6, L, *b*) have grown down to make contact with the disorganized cells. Perhaps the outer air, filtering through the dead cells, served as the stimulus inciting this growth.

In Plate 6, M (drawn at higher magnification than the preceding), a hypha has grown down through a cell of the lower epidermis and its tip has flattened against the outer epidermal wall. There are two small nuclei (pl. 6, M, *a*) near the tip. Hyphae of this sort also are regarded as receptive hyphae.

GREENHOUSE DATA ON HETEROTHALLISM

Although infections of *Puccinia coronata* may grow faster or slower according to local conditions, the general course of development is similar in all, up to the maturity of the spermogonia. From then on there is a difference. Some of the mycelia produce fertile aecia. Others remain sterile, i. e., produce no aeciospores.

An attempt was made to determine whether this difference is due to heterothallism. Infections are classified as single (not in contact with others), double (two growing in contact), and multiple (several infections confluent). In a heterothallic rust with two sexual strains, if the infections are left undisturbed and spermatia are not transferred from one infection to another by outside agencies, it would be expected that all the single infections and 50 per cent of the double infections would remain sterile, while the other 50 per cent of the double and nearly all the multiple infections would produce aeciospores.

In a preliminary test, a much higher percentage of the infections developed aecia than would be expected. The inoculated plants were covered with tarlatan cages, but it was discovered that minute yellow insects not excluded by the cages were going from one infec-

tion to another, feeding on the spermogonial exudate and incidentally transferring spermatia from one infection to another. As a result, out of 248 single infections, 37 per cent had developed open aecia by the twentieth day after inoculation, 71 per cent by the thirtieth day, and 83 per cent a week or so later. All the multiples bore open aecia by the twentieth day. The effectiveness of insect transfer, at least, is amply demonstrated.

In a second experiment some of the inoculated plants bore only one infection, and from others all but one were removed. These plants were carefully isolated. The results are tabulated separately. (Table 1.) Nearly all the material was fixed between the thirtieth and the fiftieth day. Comparatively little of it reached the age of 50 days.

TABLE 1.—Percentage of infections of *Puccinia coronata* with open aecia at different ages

[1930 material]

Group	Infections of indicated age										Infections with open aecia expected
	20 days			30 days			30 to 50 days				
	Total	With open aecia		Total	With open aecia		Total	With open aecia			
Singles.—One infection on a plant	Number 8	Number 0	Per cent 0	Number 8	Number 1	Per cent 12	Number 8	Number 1	Per cent 12	Per cent 0	
Singles.—Several infections on a plant	79	8	10	^a 65	16	25	63	18	29	0	
Doubles	6	3	50	^b 5	3	60	5	3	60	50	
Multiples	3	1	33	3	3	100	3	3	100	(^c)	

^a 2 sterile infections died, and several others were fixed.

^b 1 fertile infection was fixed.

^c Nearly all.

There is evidence here of heterothallism. Eight of the *Rhamnus* plants bore one infection each. Of these eight infections, only one developed aecia, and it may be that, in spite of all attempts to isolate it, accidental fertilization occurred here. Where several singles occurred on one plant the chances that spermatia might be transferred by insects are proportionally increased, and this is reflected in the ratios. The number of doubles and multiples recorded in Table 1 is too small to be significant, but the evidence, so far as it goes, supports the assumption that *Puccinia coronata* is heterothallic.

On a plant bearing six infections well separated from one another, the spermatia were thoroughly mixed on the seventh day. Five of

EXPLANATORY LEGEND FOR PLATE 7

A.—Portion of unfertilized aecium from 10-day infection showing the growth of hyphae to the stoma at *a*. $\times 640$.

B.—Portion of unfertilized aecium from 10-day infection showing longitudinal section of stoma at *a*, and the hyphae above centered upon it. $\times 640$.

C.—Detail from 8-day unfertilized infection: *a*, Probably remnant of subcuticular growth; *b*, decadent intracellular hypha from subepidermal hypha (*c*). $\times 1,020$.

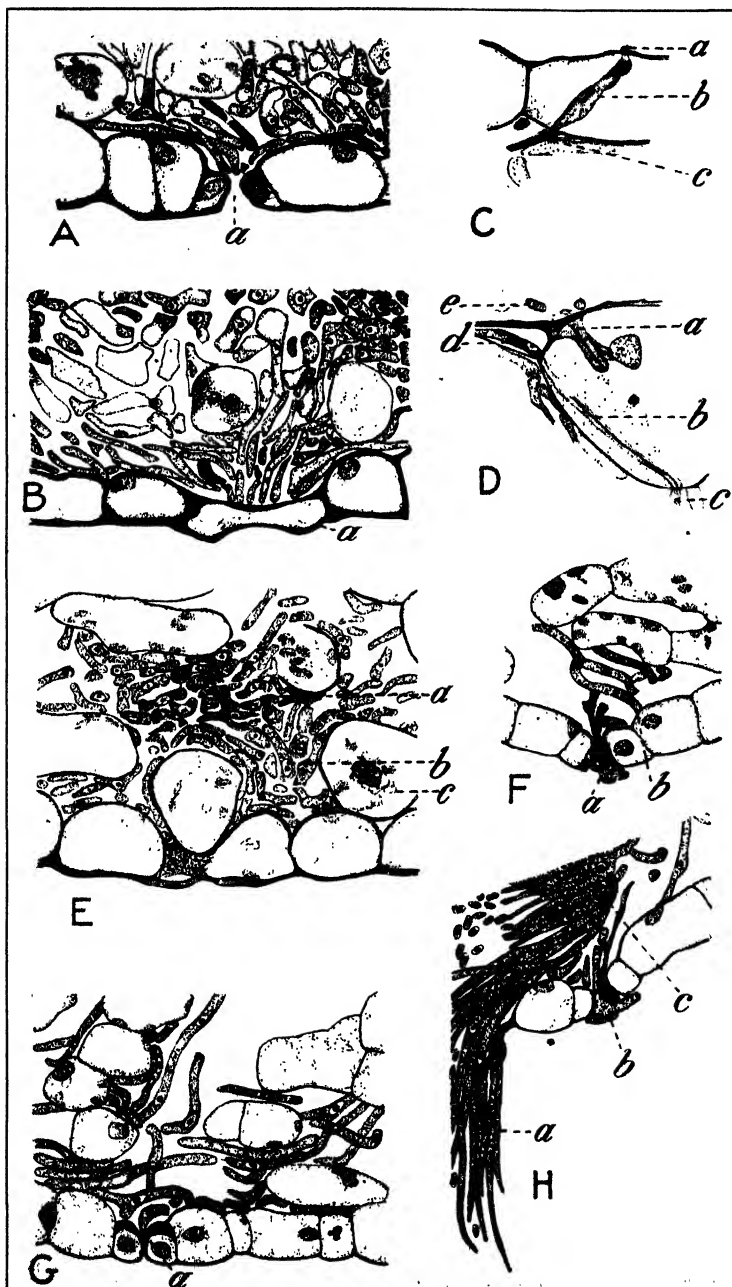
D.—Bit of upper epidermis of 8-day sterile infection near spermogonium: *a*, Heavily insheathed remnant of a haustorium; *c*, subepidermal hypha that grew through the epidermal cell (*b*) and on to form a hypha (*d*); *e*, spermatium. $\times 1,020$.

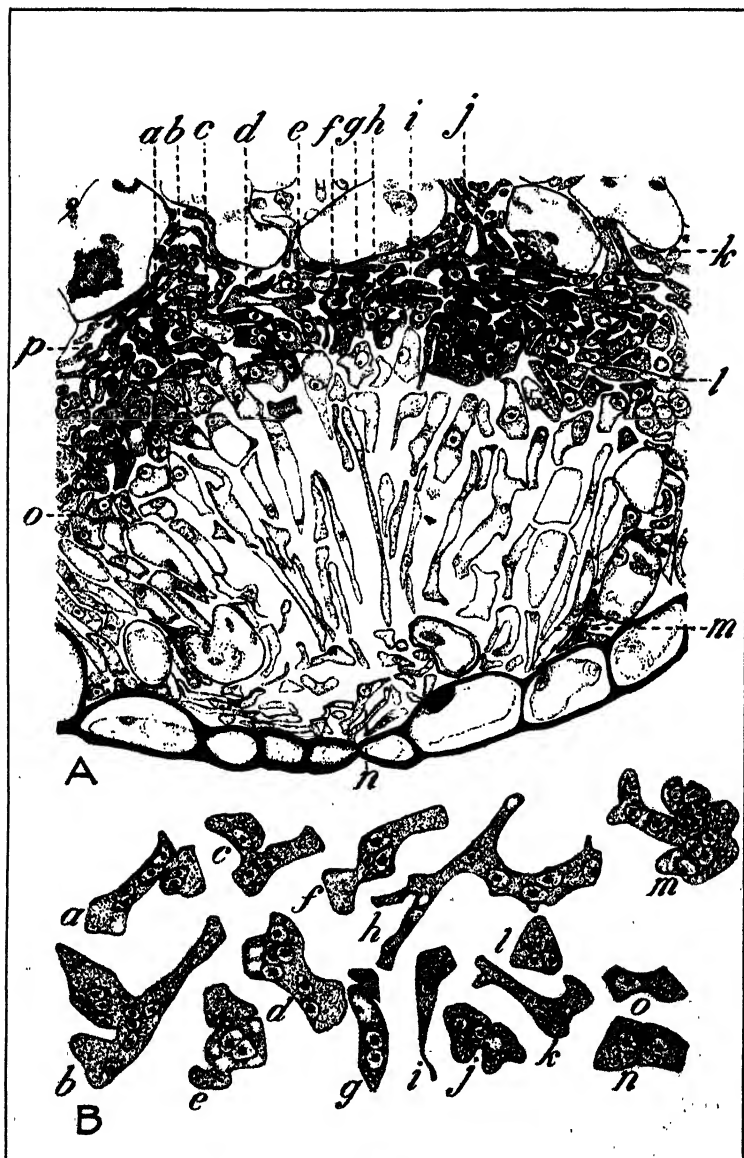
E.—Young sterile aecium: *a*, Upper half, of small dense cells; *b*, lower half, of larger cells; *c*, dead host cell. $\times 640$.

F.—Eight-day sterile infection: *a*, Stoma filled with receptive hyphae from vegetative mycelium (*b*). $\times 640$.

G.—Beginning of aecium in 8-day infection: *a*, Young receptive hyphae at stoma. $\times 640$.

H.—Portion of spermogonium at lower surface of leaf in 8-day sterile infection: Paraphyses (*a*) and receptive hyphae in stoma (*b*) have grown from the margin of spermogonium at *c*. $\times 640$.





the six infections produced fertile aecia. This fact, combined with the fact that seven out of eight of the best isolated infections bore no aeciospores, affords strong evidence that spermatia of *Puccinia coronata* function in initiating the sporophyte generation. The results as a whole show clearly the agency of insects in transferring the spermatia.

THE UNFERTILIZED INFECTION

In the isolated infection the vegetative mycelium, so far as noted, is composed of uninucleate cells. Aecia form regularly, develop up to a certain stage, and then deteriorate.

The sterile aecium (pl. 7, G, drawn from an 8-day infection) begins as a small cluster of hyphae in the large intercellular spaces of the spongy mesophyll near the lower epidermis. At first it is distinguishable from the general mycelium only by the closer grouping and denser contents of the hyphae. In *Puccinia coronata* the aecial primordium is not necessarily initiated about the base of a receptive hypha at a stoma. The aecium sometimes comes first and the receptive hyphae later. In Plate 7, G, the aecium is well begun, but the only indication of receptive hyphae is the young hypha (*a*) in the inner angle between the two guard cells of a shut stoma. There is no fully formed receptive hypha connected with this young aecium.

Although an aecium may be well started without having any receptive hypha directly connected with it, there is usually an abundance of hyphae not far away. In the 8-day infection from which Plate 7, G, is drawn there are stomata filled with hyphae. (Pl. 7, F, H.) In Plate 7, F, three or four hyphae in turn have forced themselves into the stoma at *a*. These receptive hyphae took their origin in ordinary vegetative hyphae above, at *b*. In Plate 7, H, the occupied stoma lies close to the ostiole of a spermogonium. The paraphyses at *a* and the receptive hyphae at *b* have a common origin in cells of the side wall of the spermogonium near *c*. In both cases (pl. 7, F, H) the first hyphae to enter the stoma have died, and only the latest entrants are still living.

By further growth and branching within the area of the young aecium, the hyphae form a dense little nest. Cells within the lower half of this nest soon begin to expand. An early stage of this differentiation of the young aecium into upper and lower halves is represented in Plate 7, E. At *a*, the tightly snarled hyphae are small and dense and closely septate. Lower, at *b*, the cells are somewhat larger and their contents more vacuolate.

Even at this stage in the development of the aecium (pl. 7, E) there may be no surface hyphae directly connected with it. A careful examination of the sections from beginning to end of this young aecium reveals no receptive hyphae in stomata, between epidermal cells, or growing out intracellularly through epidermal cells. The only hyphae that could possibly be considered receptive are some that have grown down to make contact with a dead, shrunken epidermal cell in an adjoining section.

EXPLANATORY LEGEND FOR PLATE 8

A.—Median section through aecium of 10-day sterile infection: *a* to *f*, Numerous cells, each with two or three nuclei in the upper arch of denser cells (*k*, *l*, *o*, *p*); the open space-making layers below (*i*, *m*, *n*, *o*) are uninucleate. $\times 640$.

B.—Cells from aecia of a sterile 10-day infection: *a* to *m*, Representative multinucleate cells; *n* and *o*, typical uninucleate cells. $\times 1,020$.

It may be that air, entering the leaf from without, whether it enters through a dead cell or through a stoma, exerts an attractive influence on the fungus. Hyphae will grow to a stoma whether that stoma adjoins a spermatogonium, ordinary mycelium, or an aecium. Plate 7, A and B, show hyphae growing to stomata adjoining sterile aecia. In Plate 7, A, *a*, the stoma is shown in cross section, and in B, at *a*, the stoma is cut longitudinally. In neither is the stoma opposite the center of the aecium. In both, hyphae have grown in from all directions toward the stomatal aperture but have not actually entered it.

Reference was made earlier (pl. 6, E, M) to intracellular hyphae that push out through an epidermal cell and sometimes pierce the outer epidermal wall and grow on subcuticularly. When they fail to make contact with spermatia, these receptive hyphae, like those in the stomata, soon die. In Plate 7, C, the subepidermal hypha (*c*) gave rise to the intracellular hypha (*b*), which grew through the epidermal cell to its outer wall. At *a* is a minute object which may be the dried remnant of a former subcuticular hypha. The intracellular growth is dead. Plate 7, D, shows the dead intracellular hypha (*b*) coming from the mycelial hypha (*c*). This epidermal cell lies at the edge of a spermatogonium, and the hypha (*b*) issued into an adjoining crushed, empty epidermal cell at *d*. At *a* is a dead and heavily in-sheathed structure, apparently the remnant of a haustorium entering the cell from earlier subcuticular growth.

As the haploid mycelium spreads through the host leaf, new aecia form in succession in the younger marginal growth. A section through the center of a well-grown, 10-day, unfertilized infection shows all stages in the development of aecia from the early stages in the margin to full-grown and degenerating sterile aecia in the center. The young haploid aecium shown in Plate 7, E, is found near the edge of such an infection; the larger sterile aecium shown in Plate 8, A, occurs farther in; while at the center of the same infection are full-grown aecia showing degenerative changes characteristic of older aecia when fertilization fails to take place. No indication of fertilization was noted at any point in the infection.

In the aecium shown in Plate 8, A, referred to above, the differentiation into upper and lower areas is completed. The lower area of vacuolated cells in the aecium has expanded into the shape of a very thick, biconvex lens. (Pl. 8, A, *l-m*.) The cells of this looser or "space-making" tissue (pl. 8, A) are no longer increasing in number but are elongating radially, presenting in section a fan-shaped arrangement centered at *n*. These cells are still uninucleate and loosely spaced; many are empty and quite transparent. The area of denser cells (pl. 8, A, *k, l, o, p*) that caps the space-making tissue is composed of five or six layers of short, thick, often quite irregular cells. The majority are uninucleate, but in the central region (pl. 8, A, *a-j*) there are a number of cells containing two or three nuclei. As stated above, this aecium is quite certainly unfertilized, for all the older aecia of the same infection (Table 2, 10-day) show marked evidence of degeneration, yet it contains several dozen binucleate or trinucleate cells.

Ordinarily, from the tenth day on, multinucleate cells are regularly present in sterile aecia. It has been assumed hitherto that the presence in an aecium of cells containing more than one nucleus means sporophytic growth which will lead to spore formation. The

finding of multinucleate cells in nearly 100 per cent of the older sterile aecia of *Puccinia coronata* is a point of theoretic interest, and a detailed study has been made of these sterile infections of different ages.

The question arises as to the mode of origin of these binucleate and trinucleate cells. Some of them (pl. 8, A, *e, f, h*) are highly irregular in form. Their unexpected angles, clefts, and branches may be the result of either the fusion of two or more cells or the irregular growth of one. Others (pl. 8, A, *b, c*) are simple in form and give no suggestion from their shape that they are the product of recent fusion.

Plate 8, B, *a* to *m*, shows, at high magnification, representative multinucleate cells from sterile aecia of this and slightly later stages, with a few typical uninucleate cells (*n, o*) for comparison.

No great insight as to the mode of origin is to be gained by a study of these cells. In some cases an origin by fusion seems possible, for in spite of the growth that the cells may have made since fusion, they still suggest by their shape a double origin. Where there is a relatively narrow isthmus between two parts of a cell (pl. 8, B, *a, b, c, e, f, g, j*) there is a possibility of origin by fusion. Even here, however, there is the alternative possibility of the irregular growth of a single cell constrained into irregular forms by the pressure of adjoining cells. One point seems clear—it can not be assumed that multinucleate cells are the result of multiple fusion. Cells (pl. 8, B, *b, m*) that contain 10 or 12 nuclei more probably have arisen by simple growth and nuclear divisions or possibly by a single initial fusion followed by such growth.

Soon after the stage of development represented in Plate 8, A, sterile aecia begin to degenerate. Plate 9, A, shows a semidiagrammatic section through a sterile 16-day infection. The leaf is considerably hypertrophied, as may be seen by comparing its thickness at the center and at the ends of the drawing; the whole infected area is arched upward, making a raised spot on the upper surface of the leaf.

The younger aecia of the series, located either in the fresh young marginal growth (pl. 9, A, *a, f*) or in a belated development on the upper surface (pl. 9, A, *g, h*) are vigorous in appearance and resemble closely the aecium shown in Plate 8, A. The older aecia (pl. 9, A, *b, c, d, e*) are showing signs of degeneration.

The cells of these older sterile aecia are somewhat impoverished and take a diffuse stain, although comparatively few of the aecia in this case contain dead cells. At this stage each of the larger aecia has pushed the lower epidermis outward into a convex curve. (Pl. 9, A, *b, d*.) Later, the sterile aecium shrinks and may even be marked by a depression of the surface.

A summary of the condition of aecia in sterile infections of *Puccinia coronata* of different ages has been made. In this study (Table 2) the aecia of each infection are classified as (1) too young to show multinucleate cells, (2) containing living multinucleate cells, (3) sterile and containing both living and dead multinucleate cells, and (4), in a few cases, cells with two to four nuclei and spores. Counts were made in infections of different ages and the data tabulated. Each horizontal line (Table 2) represents the aecia of one infection.

TABLE 2.—Summary of condition of aecia in sterile infections of *Puccinia coronata* of different ages

[1928 material]

Age of infection (days)	Number of aecia showing indicated condition *				Age of infection (days)	Number of aecia showing indicated condition *			
	Too young to show multinucleate cells	Containing multinucleate cells living	Containing both living and dead multinucleate cells	Containing multinucleate cells and spores		Too young to show multinucleate cells	Containing multinucleate cells living	Containing both living and dead multinucleate cells	Containing multinucleate cells and spores
10	14	13	9		16	5	13	8	
11	7	8			16	30	27		
11	3	20			17	10	4		
12	4	4			17	13	19	32	
12	8				17	5	18	19	
13	10	4		1	18	3	11	5	1
13	23				18		11	4	
13	15	10			23	1	20	18	
13	16			1	26	1	6	6	
14	1	4			26	2	6	15	
14	25	2			26	13	15		
14	10	3			28		16	8	
14	9	4			30		1	35	
15	8				30			41	
15	1		3		62			20	
16	17	2			62			12	
16	2	18	10						

* Leaders indicate that aecia were not found.

From Table 2 it will be seen that the aecia of a single infection present a broad range of development, for during a considerable period new aecia keep forming in the fresh marginal growth of the infection. There is also considerable variation between infections of the same age. This is due partly to differences in the environment, for infections grown late in the spring and thus exposed to higher temperatures and stronger light develop faster than do those of early spring. The variation may also be due to localized differences within the host tissues, for of two infections of the same age growing side by side on the same leaf, one may develop faster than the other.

In three of the infections included in the study (Table 2) a single aecium developed spores. In such cases, when the infection is young enough, the sporophyte generation can spread farther. In greenhouse studies, however, it is not rare to find an old decadent infection with a single open aecium. This is perhaps explained by the fact that the transfer of spermatia by insects has taken place so late that only one aecium is still young enough to respond.

In general, up to the fifteenth day (Table 2) over half of the aecia are too young to have developed multinucleate cells, and where they do occur these multinucleate cells are still living. Beginning with infections 16 days old, the proportion of young aecia decreases and the proportion with living multinucleate cells increases. In the later

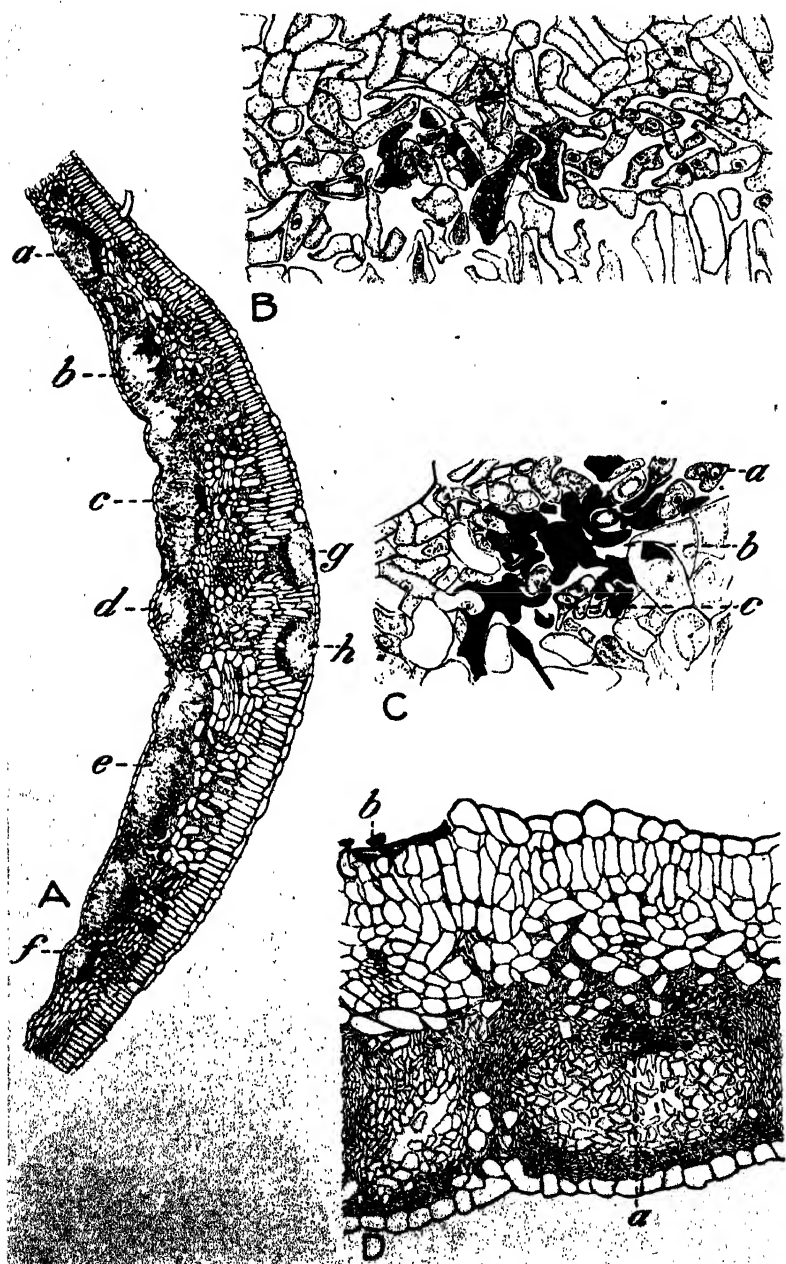
EXPLANATORY LEGEND FOR PLATE 9

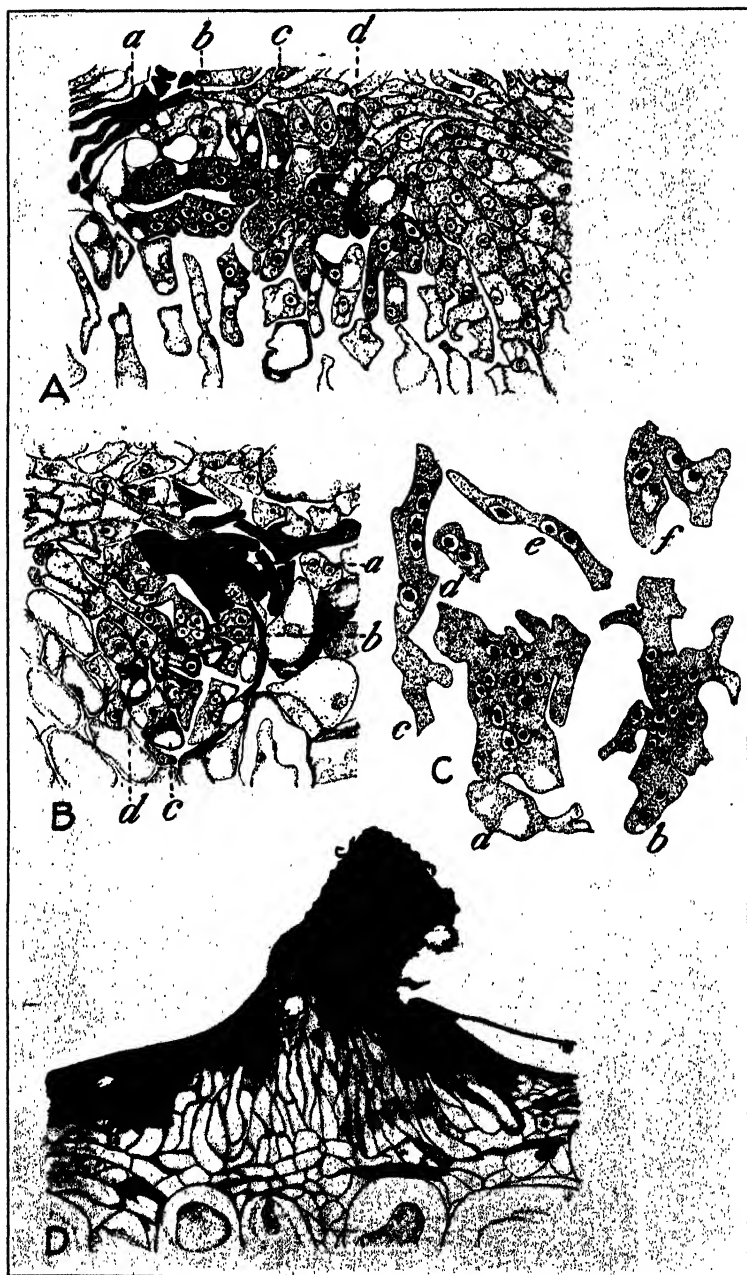
A.—Diagram of section through a 16-day sterile infection: *a*, *f*, *g*, and *h*, Younger aecia; *b*, *c*, *d*, and *e*, older aecia. $\times 45$.

B.—Detail from upper central part of aecium from 23-day sterile infection, showing deteriorating multinucleate cells. $\times 640$.

C.—Twenty-three-day sterile infection: *b*, Dead multinucleate cells; *a* and *c*, living multinucleate cells. $\times 640$.

D.—Diagram of sterile aecium of 26-day infection: *a*, Dead multinucleate cells; *b*, denuded leaf surface. $\times 115$.





history of the sterile infections (Table 2, 18-day to 62-day) there is a steady decrease in the number of young aecia and later a decrease in the number of those whose multinucleate cells are all living, until finally all the aecia are in the third class, i. e., they contain both living and dead multinucleate cells.

A similar study was made of sterile infections in the rust grown in 1930. Here, as before, multinucleate cells were of regular occurrence in older sterile aecia, but in these slower-growing infections the multinucleate cells did not appear before the twenty-fifth day after inoculation, fully two weeks later than in the Canadian rust used in 1928. Once formed, however, the multinucleate cells followed the same course of slow growth, nuclear multiplication, and subsequent deterioration. A few multinucleate cells were still living in an infection 49 days old.

Plate 9 shows details of these degenerative changes within the sterile aecium. A group of multinucleate cells (pl. 9, B) shows the beginning of degeneration in a 23-day infection. The number of nuclei in a cell varies from two to eight. Degeneration is evidenced by the dimness of detail in these cells and a decided tendency to stain red with safranin. As the cells around this group are impoverished in content and stain very faintly, the contrast between the two is sharp and can be seen clearly even with the low power of the microscope.

In this same infection are other aecia in all stages of degeneration. In Plate 9, C, nearly all the multinucleate cells are dead and densely stained with red. Only two binucleate cells (*a*, *c*) are left alive.

Something of the appearance of the sterile aecium a little later (after 26 days) is shown in Plate 9, D. Both the fungus and its host have increased greatly in size. In the upper half of the aecium at *a* is a group of dark-stained dead multinucleate cells. Multinucleate cells, living or dead, are ordinarily located in the upper central part of the aecium (pl. 9, D, *a*), but in older aecia they are sometimes separated into patches and may be erratically placed near the side walls of the aecium, or even out in the space-making tissue. It is probable that they all originate as figured in the younger aecia (pl. 8, A) but become displaced by subsequent localized growth in adjoining areas of the aecium.

Among the dead multinucleate cells are usually a few survivors, which may grow further. In Plate 10, A, between the dead or dying cells at *a* and *d* are several large living multinucleate cells. One of them (*c*) is irregularly lobed and branched; another (*b*) suggests by its shape that it may be a product of fairly recent fusion. In Plate 10, B, is another group, also from a 26-day infection. Beside a group of dead cells (*a*) is a nest of living multinucleate cells (*b*). Some of these cells look vigorous, but others (*c*, *d*) are dying.

Sometimes one of these sterile infections, if very favorably placed, may live on for eight or nine weeks. Within its aecia are still to be found a few living multinucleate cells. Plate 10, C, shows such cells from a 62-day infection. Some are small and contain few nucle

EXPLANATORY LEGEND FOR PLATE 10

A.—Multinucleate cells in aecium of 26-day sterile infection: *a* and *d*, Dead or dying cells; *b* and *c*, large living cells. $\times 640$.

B.—As in A: *a*, Dead cells; *c* and *d*, dying cells; *b*, living cells. $\times 640$.

C.—Living multinucleate cells (*a*, *b*, *c*, *d*, *e*, *f*) from an aecium in a sterile 62-day infection. $\times 1,020$

D.—Dead spermatogonium from a 23-day sterile infection. $\times 640$.

(pl. 10, C, *c*, *d*, *e*, *f*), others have grown and branched irregularly (pl. 10, C, *a*, *b*) and may contain as many as 20 nuclei. The age and mode of origin of cells like these are matters of conjecture only.

The spermogonial exudate in *Puccinia coronata* is never abundant, and the spermogonia for the most part are short lived. The spermogonia in these sterile infections do not, as in *P. graminis*, continue to function throughout the lifetime of the fungus. They soon die. In one 23-day infection no functioning spermogonium remains. Plate 10, D, shows the condition of the spermogonium. It has already been noted that the spermogonia form on a continuous cushion or stroma beneath the epidermis. Before the sterile infection is very old, sometimes as early as the fifteenth day, this stroma begins to be sloughed off, leaving the palisade of the leaf tissue exposed. The edge of one of these denuded areas is shown in Plate 9, D, at *b*.

FERTILIZATION AND THE FERTILE AECIUM

A monosporidial infection bears both spermogonia and receptive hyphae, but the spermatia borne by an individual apparently can not start the sporophyte generation in that same mycelium, for when an infection is carefully isolated it remains sterile and produces no aeciospores. But when spermatia are transferred to infections of opposite sex, the sporophyte generation is initiated and fertile aecia form.

When spermatia are transferred either by insects or by laboratory methods they are applied to the leaf surface (either upper or lower) at the infected area. Spermatia scattered along the surface of the epidermis can enter at any point where the mycelium reaches the surface.

Plate 11, C, shows, at low magnification, part of a section through a 12-day infection. At *c* is one spermogonium and at *a* a lateral section through a second. There are sporophytic cells between the two at *b*; this area is shown enlarged in Plate 11, A.

In Plate 11, A, a hypha (*e*) of the subepidermal mycelium grew into an epidermal cell at *d*, and out through the outer wall to form a subcuticular hypha (*c*). The connection between *c* and *d* is found in the next section. At *a*, on the surface of the leaf, are spermatia. At *b*, where hypha and spermatia meet, there is a break in the cuticle and three small dark masses (possibly of spermatial origin) are embedded in the end of the hypha. That spermatial nuclei entered the hypha and passed down into the inner mycelium seems fairly certain, for binucleate cells are seen in the subepidermal region at *f*, *g*, and *h*. Both the intracellular hypha and the subcuticular hypha are now decadent. At *i*, within the body of the spermogonium, are two binucleate cells. It does not seem probable that sporophytic hyphae originating outside a mature spermogonium would later grow into it.

EXPLANATORY LEGEND FOR PLATE 11

A.—Detail from C enlarged: *a*, Spermatia; *b* and *c*, subcuticular hyphae; *d*, intracellular hypha from subepidermal hypha (*e*); *f*, *g*, *h*, and *i*, sporophytic cells. $\times 640$.

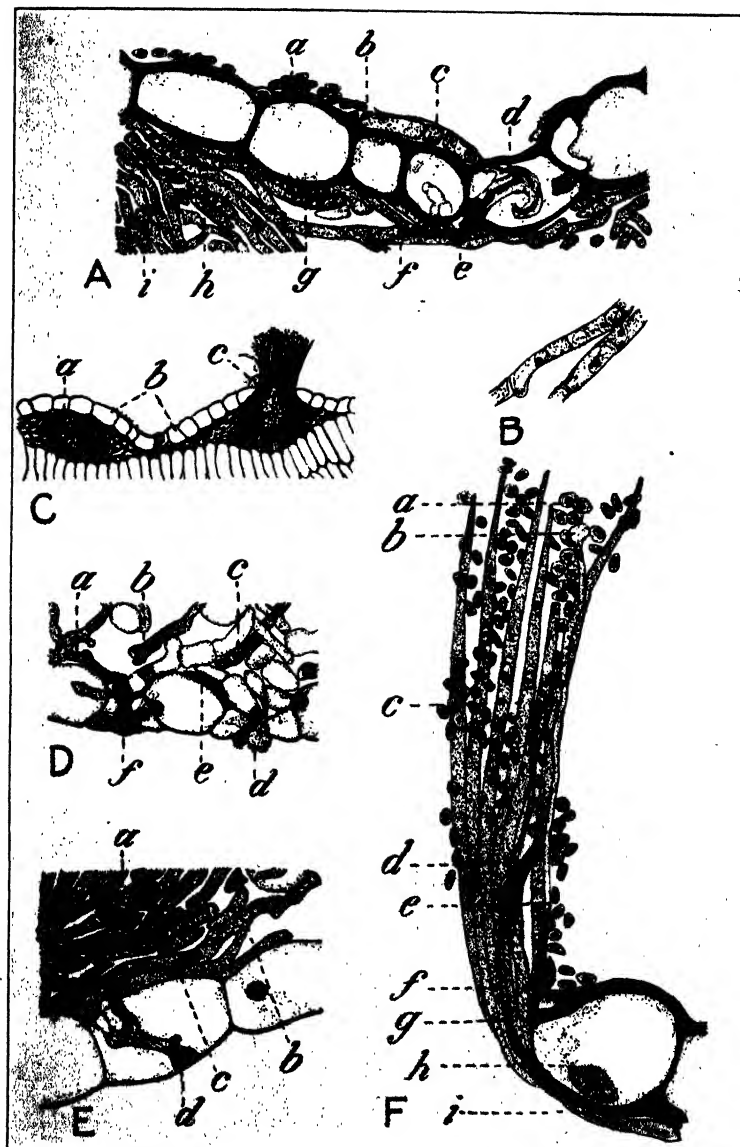
B.—Detail of mycelium of 13-day infection showing two hyphae tangent and fused at the point of tangency. $\times 1,020$.

C.—Diagram of subepidermal web of 12-day infection: *a* and *c*, Spermogonia; *b*, detail enlarged in A. $\times 115$.

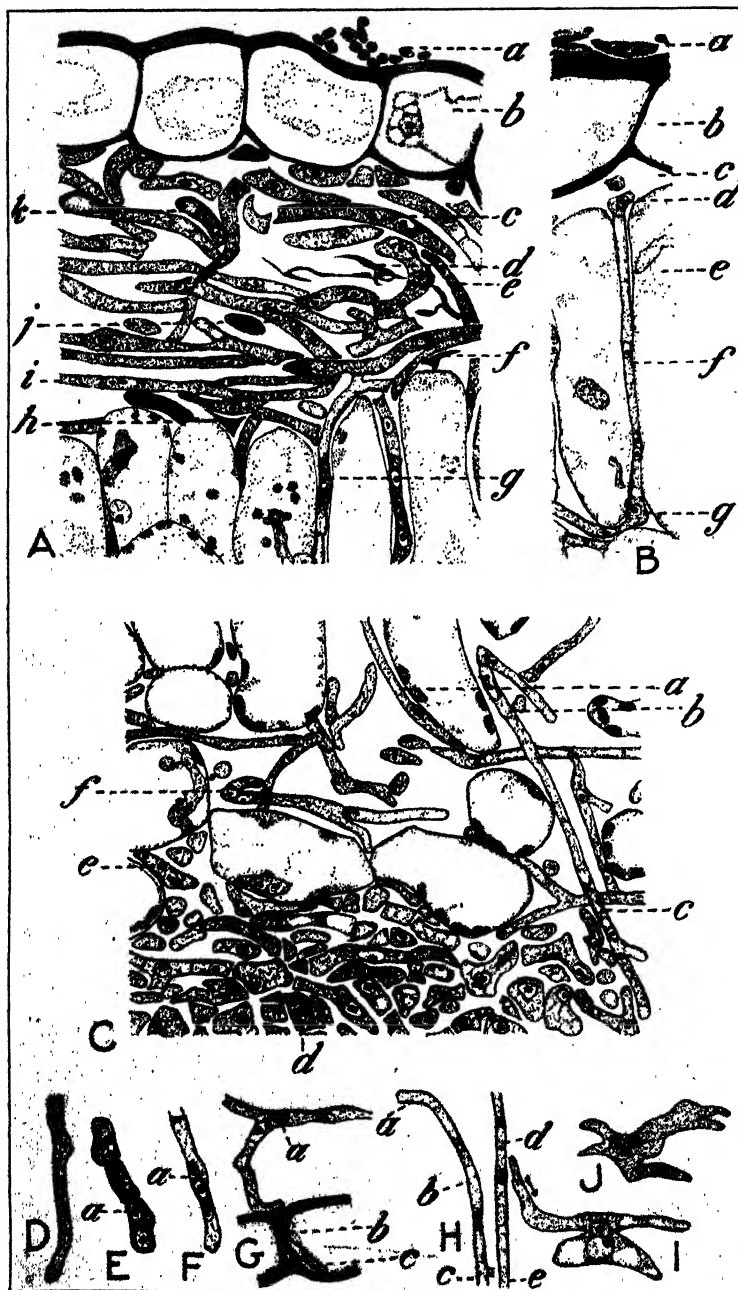
D.—Detail from lower leaf surface of 8-day infection: *d* and *f*, Stomatal hyphae; *a*, *b*, *c*, and *e*, sporophytic hyphae above. $\times 640$.

E.—Seven-day infection: *a*, Part of spermogonium; *b* and *c*, young paraphysis giving rise to an intracellular branch (*d*) that has grown out through the epidermal cell; the branch contains six nuclei. $\times 1,020$.

F.—Portion of tuft of paraphyses of a spermogonium, with adherent spermatia, from 12-day infection: *a*, Expanded spermatia; *b*, germinating spermatium, with germ tube leading to *e* and *g*; *c*, second germ tube, leading to *f* and *i*; *d*, decadent paraphysis; *h*, epidermal cell. $\times 1,020$.



FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE



It is possible that spermatial nuclei entered at a second point somewhere within the spermogonial area.

Plate 11, D, from an 8-day infection, shows another possible method of entrance. Here are two adjoining stomata in the lower epidermis, one (*f*) cut across the guard cells, the other (*d*) parallel to them. In both stomata are hyphae filling the stomatal aperture, and above, at *a*, *b*, *c*, and *e*, are binucleate or trinucleate fungus cells. The finding of sporophytic cells next to a stoma is not in itself proof that the nuclei entered at that stoma, for the sporophytic hyphae might have grown in from some other point. The likelihood is, however, that fusions frequently take place at stomata in the lower surface of the leaf.

Reference was made earlier (pl. 6, M) to the frequent occurrence of intracellular hyphae that grow through the epidermal cell to its outer wall and stop there, without forming subcuticular growth. The example shown in Plate 11, E, suggests that these also may serve to admit spermatial nuclei. The present instance occurred at a young spermogonium at the lower epidermis of a 7-day infection. A branch from a young paraphysis (*b*, *c*) grew out intracellularly through an epidermal cell and flattened itself against the outer epidermal wall at *d*. No opening can be seen in the host cell wall, but the hypha (*d*) contains six minute nuclei, and the paraphysis (*c*) of which it is a branch contains three full-grown nuclei. The inference is that there were spermatia on the lower leaf surface (later washed off during fixation or dehydration) and that the extremely small spermatial nuclei passed through the wall of the epidermal cell into the hypha. The trinucleate cell at *a* in the interior of the spermogonium remains unexplained.

It is with some hesitation that Plate 11, F, is added to the list of possible modes of entrance, for it differs markedly from the rest. It shows part of the brush of paraphyses of a spermogonium from a 12-day infection. At *h* is an epidermal cell of the leaf, and from *a* to *g* the extruded part of a group of paraphyses with the adherent exudate. Some of the spermatia (perhaps introduced spermatia) have expanded, rounding out from their original capsule shape to a sphere (*a*). One of these swollen spermatia at *b* has pushed out a fine germ tube, probably utilizing the sirup of the exudate as food, and the tube has grown down along the surface of a paraphysis to *e*, and on to *g*, where it passes behind other cells. At *c* is a similar germ tube, the upper end of which was removed in sectioning. It can be traced from *c* down to *f* and on to *i* (inside the host plant), beneath the epidermal cell. It contains a nucleus at the growing tip at *i*. At *d* there is a coarser structure,

EXPLANATORY LEGEND FOR PLATE 12

A.—Portion of subepidermal web of 12-day infection: *a*, Spermatia on upper epidermis (*b*); *c*, *e*, *i*, *j*, and *k*, sporophytic cells in the mycelial web; *d*, *f*, and *h*, remnants of earlier hyphae; *g*, trinucleate hypha between palisade cells. $\times 1,020$.

B.—Twelve-day infection: *a*, Spermatia on epidermis (*b*) at outer margin of subepidermal space (*c*); *d*, *f*, and *g*, successive cells of hypha with 2, 3, and 2 nuclei, respectively; *e*, palisade. $\times 1,020$.

C.—Thirteen-day infection: *a*, *b*, *c*, *e*, and *f*, Sporophytic mycelium in air spaces of mesophyll above acium; *d*, binucleate cell in acium. $\times 1,020$.

D.—Sporophytic hypha, with 2-1-3 nuclei in successive cells, from 13-day infection. $\times 1,020$.

E.—Hypha, from sporogenous area of acium of 13-day infection, with 2-2-3 nuclei in successive cells. $\times 1,020$.

F.—Mycelial cell of 13-day infection, with two large and two small nuclei: *a*, cell with large and small nuclei. $\times 1,020$.

G.—Detail of 13-day infection: *b*, Hypha between epidermal cells and intracellular branch (*c*); *a*, continuation of same hypha above, with four nuclei in one cell. $\times 640$.

H.—Thirteen-day infection: *a*, *b*, *c*, *d*, and *e*, Hypha found between palisade cells; the successive cells have 4, 0, and 3 nuclei, respectively. $\times 1,020$.

I.—Fusion cell from sporogenous area of acium of 13-day infection. $\times 1,020$.

J.—Multinucleate cells from acium containing fusion cell shown in I. $\times 1,020$.

probably an abnormal or decadent paraphysis. These germ tubes are extremely minute (one-third to one-half the diameter of a paraphysis), and only when they are sharply stained can they be seen at all. In safranin and in methylene blue the paraphyses tend to stain pink and the germ tubes blue. Of course it is possible that these are minute spores of some other fungus that alighted accidentally on spermogonial exudate and started to grow there, but no other evidence of secondary fungi has been seen. Moreover, the enlarged spermatia at *a* are evidence against that theory. If when introduced spermatia adhere to paraphyses (as must frequently happen) they can grow down through the ostiole and combine there with native hyphae, it would go far to explain the rather frequent occurrence (pl. 11, A, *i*; E, *a*) of binucleate or trinucleate cells in the body of the spermogonium and along its walls.

Plate 11, B, shows still another possibility. When two infections of opposite sex are located close together in the leaf and become confluent as they grow, open aecia are formed on both infections. In such cases the diploid growth may start by means of the two kinds of spermatia on the leaf surface, and probably does so. Plate 11, B, suggests that the sporophyte generation may also originate within the leaf by hyphal fusions. The two hyphae (pl. 11, B) are tangent; at the point of contact there is an open passageway from one to the other, and the nuclei of the two cells are in contact. The infection is a little too old (13 days) for a determination of its multisporeidial origin by means of the primary hyphae, for the latter have so far degenerated that they can not be distinguished with certainty from haustoria and intracellular receptive hyphae, which also occur in epidermal cells. The grouping of the spermogonia, however, strongly suggests two centers of growth, and the probability is that two infections overlapped here.

A branched hypha (pl. 15, A, *a*), found beside a young aecium in another infection, is doubtful; but it, too, is at least open to interpretation as a hyphal fusion.

By whatever method the sporophyte starts, it soon spreads from the point or points of origin; and occasional hyphae with cells of more than one nucleus are found scattered among the haploid hyphae.

As previously stated, when spermogonia in the young infection form on the subepidermal mat of hyphae, they lift the epidermis well above the palisade, leaving an open space in the leaf in the intervals between spermogonia. The hyphae of this open space are often stretched and broken in this process and usually die. (Pl. 5, B and D.) This space, however, soon fills with secondary growth.

Since the sporophyte generation may start at various points along either the upper or lower epidermis, it naturally spreads from those points in the comparatively open spaces of the subepidermal web of mycelium. Plate 12, A, shows a portion of this subepidermal growth.

EXPLANATORY LEGEND FOR PLATE 13

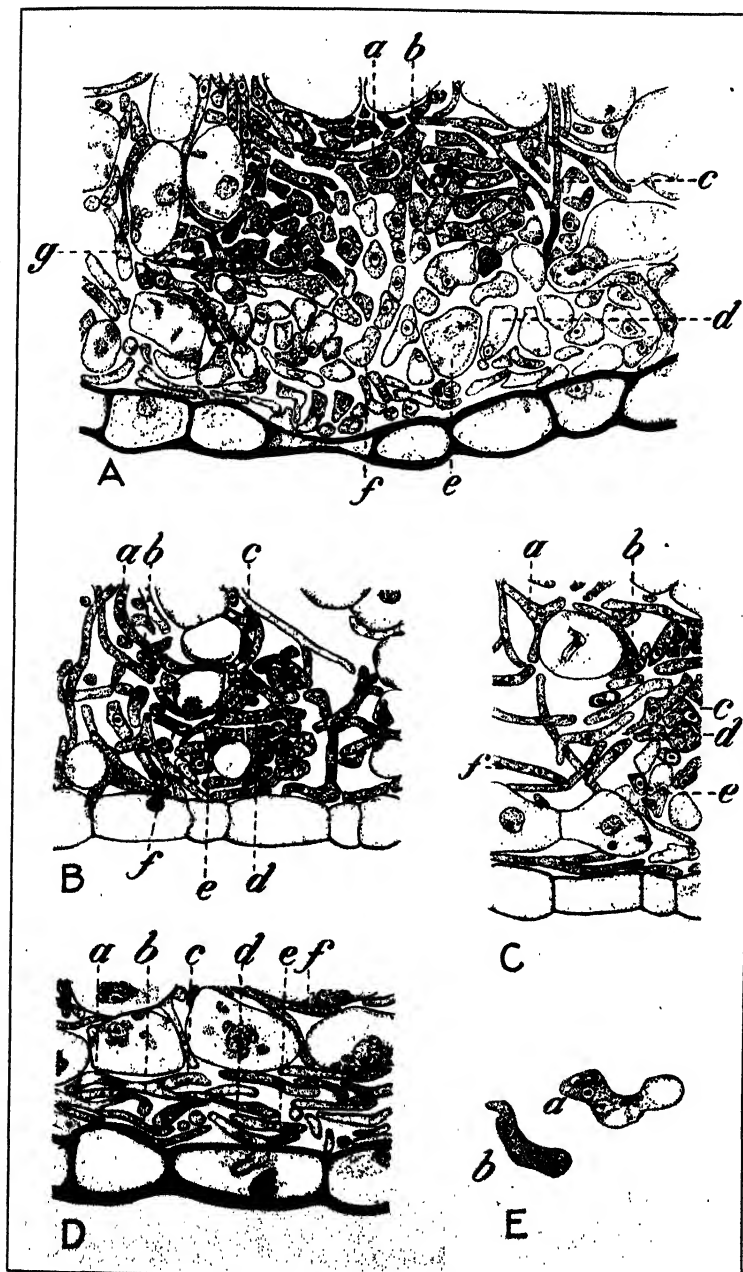
A.—Aecium of uninucleate cells: *a* and *c*, upper part, of denser cells; *d*, lower part, of large vacuolated cells; *b* and *f*, extremities of hypha extending through both areas; *e*, dead host cell; *g*, marks location of Plate 13, C. $\times 640$.

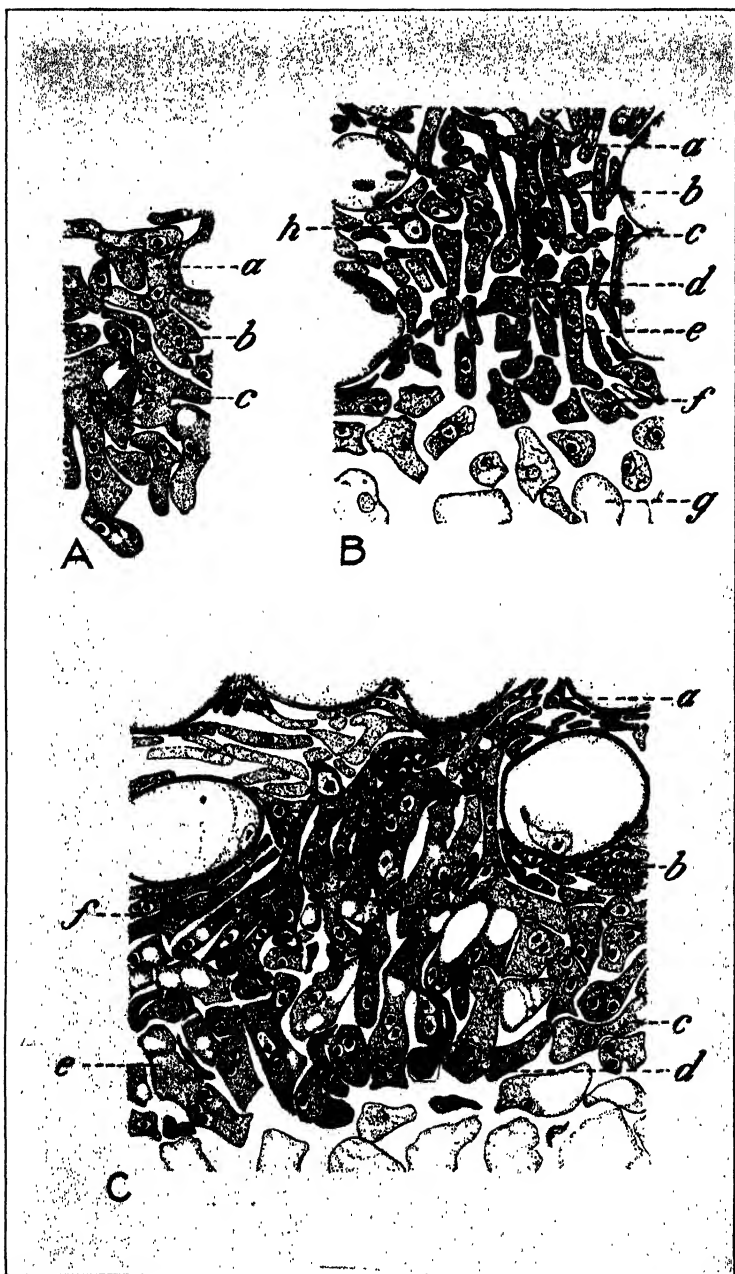
B.—Beginning of fertile aecium in 8-day infection: *a*, *b*, *c*, *d*, *e*, and *f*, Sporophytic cells. $\times 640$.

C.—Detail from section adjoining A, at *g*: *a*, *b*, *d*, *e*, and *f*, Sporophytic mycelial cells; *c*, edge of aecium. $\times 640$.

D.—Portion of subepidermal web above lower epidermis from 12-day infection: *a*, *b*, *c*, *d*, *e*, and *f*, Sporophytic cells. $\times 640$.

E.—Two multinucleate mycelial cells (*a*, *b*) thickened like aecial cells. $\times 1,020$.





Above the epidermis (*b*) is a group of spermatia at *a*. Below, at *g*, is the palisade layer of the leaf. Between epidermis and palisade is a broad space filled with a fresh growth of vigorous young hyphae, at least a third of which are diploid. In the interstices of this mat (*d*, *f*, *h*) are dead remnants of earlier hyphae.

Fresh hyphae grow down from the subepidermal weft, passing between the palisade cells and out into the air spaces of the spongy mesophyll beneath. The majority of these hyphae are composed of uninucleate cells. At Plate 12, A, *g*, however, is a trinucleate cell issuing from the weft. In Plate 12, B, a detail drawn from the thin marginal growth of another subepidermal mat, is a sporophytic hypha growing down from *d*, just beneath the epidermis, to *g*, in an air space below the palisade. There are dried spermatia on the leaf surface at *a*, and a section or so farther on a hypha traversing the epidermal cell to the surface evidently afforded means of entrance. Farther down in the same section, beyond the limits of the drawing, there are other diploid hyphae in mycelium above a young aecium.

Plate 13, D, shows a portion of the weft above the lower epidermis of the leaf. Here, too, is a large percentage of cells with more than one nucleus. From here, too, the sporophyte generation spreads into the air spaces of the mesophyll.

The sporophyte spreads rapidly from the point or points of origin. This can take place by ordinary growth of the diploid hyphae with a division of both nuclei at the origin of each new cell. It is probable, also, although difficult to prove, that the sporophyte can spread by the diploidization process of nuclear divisions and migrations described by Buller (?). The following data may have a bearing on this question:

It has been frequently observed that the number of nuclei in vegetative mycelial cells varies. The majority of sporophytic cells are binucleate, but trinucleate cells also are of frequent occurrence. (Pl. 12, A, *g*; B, *f*; C, *c*, *e*; H, *d*; pl. 13, C, *a*; D, *c*, *f*.) Occasionally, there are more than three nuclei in a cell. (Pl. 12, C, *f*; F, *a*; G, *a*; H, *b*; pl. 13, B, *b*; D, *e*.) Rarely, one of these multinucleate mycelial cells becomes greatly thickened (pl. 13, E, *a*, *b*), taking on the appearance of a cell in the sporogenous area of the growing aecium.

In the examples cited, the nuclei are not located in any regular way within the cell. They may be together near the center of the cell, or one nucleus may be at the end. Plate 12, H, *b*, shows one pair of nuclei at one end of the cell, another pair at the other end. Moreover, the nuclei of one cell are often of different sizes, as though they had not divided at the same time. It is fairly common to find one large nucleus and two or more small ones in the same cell. Plate 13, B, *a*, shows one large nucleus and a pair of small nuclei. Plate 13, B, *b*, shows a large central nucleus and two pairs of smaller nuclei. Plate 13, D, *e*, shows one large and three small nuclei. Plate 12, F, shows two full-grown nuclei and a pair of small ones.

EXPLANATORY LEGEND FOR PLATE 14

- A.—Detail of sporogenous area of 14-day fertile aecium. Probable fusions at *a*, *b*, and *c*.
 B.—Strip through upper half of an aecium of a 12-day fertile infection: *a*, *b*, *c*, *d*, *e*, *f*, and *h*, Sporophytic cells; *f* to *g*, looser space-making tissue.
 C.—Upper central part of 12-day fertile aecium: Haploid and diploid hyphae enter at *a*, grow down, and spread to right and left; *b*, probable cell fusion; *c* to *e*, layer of basal cells forming; *d*, spore mother cell; *f*, 3-cell hypha. (All $\times 1,020$.)

Furthermore, successive cells of the same hypha may not have the same number of nuclei. These hyphae make their way through the irregular air passages of the mesophyll, and it is seldom possible in sectioned material to trace a given hypha for more than one or two cells. Plate 13, D, shows two hyphae (*a*, *d*) with two nuclei in one cell and one nucleus in the next. In Plate 12, C, the cell at *b* has two nuclei and the next cell of the same hypha, as shown at *c*, has three. In Plate 12, B, the sequence in the hypha, as shown at *d*, *f*, and *g*, is 2-3-2. In Plate 12, D, it is 2-1-3. The hypha in Plate 12, H, is drawn in two parts from *a* to *c* and *d* to *e*, and the sequence here is 4-0-3. The middle cell in this sequence has no nucleus. This is not rare.

Another point of interest in this connection is that the septa delimiting the cells in these hyphae are frequently vague and hard to see. The end of one cell and the beginning of the next is more often recognized by a definite break in the cytoplasm than by an easily visible cross wall. This condition would facilitate nuclear migrations.

All these irregularities are difficult to explain on the basis of the apical growth of a diploid hypha with a regular conjugate division at the origin of each new cell, but are readily explainable as the divisions and migrations of one or more introduced nuclei within a ready-formed haploid hypha. Moreover, a hypha may show an irregular combination of the two processes; it may continue to grow while nuclei are moving along inside it.

If sporophytic mycelium is present when an aecium starts, the sporophytic and gametophytic components are intimately mixed in the aecium from its very inception. Plate 13, B, shows a very young aecium containing a liberal admixture of cells (*a-f*) with two or more nuclei. On the other hand, an aecium may begin in the absence of the sporophytic component and be invaded by it later on in its development. Plate 13, A, shows a vigorous young aecium composed entirely of uninucleate cells. At one edge of this aecium in an adjoining section, however, at a position corresponding to Plate 13, A, *g*, there are several sporophytic hyphae. This area is drawn separately as Plate 13, C, showing the edge of the aecium at *c*, and cells with two or more nuclei at *a*, *b*, *d*, *e*, and *f*.

Plate 12, C, already referred to, shows a similar arrangement, including the upper edge of an aecium and the mycelium in the air spaces of the mesophyll tissue above it. The mycelium includes several sporophytic hyphae composed of cells with from one to five nuclei. No direct line of sporophytic cells can be traced from the mycelium into the aecium, but one hypha (*b-c*) has reached its edge.

The aecium (pl. 12, C), which is somewhat more advanced than the one in Plate 13, A, is uninucleate throughout, except for three or four cells in the sporogenous area. These, one of which (pl. 12, C, *d*) is included in the drawing, suggest strongly by their shape that they have just been formed by the fusion of two uninucleate cells. This aecium was part of the same infection from which the hyphal fusion in Plate 11, B, was drawn. As stated before, it is probable that two mycelia of opposite sex intermingle here. It may well be that haploid hyphae of both sorts entered into the formation of this aecium and are uniting here.

Further evidence of the invasion of an aecium after it is well started is shown in Plate 14, B and C. Plate 14, B, shows a narrow

median strip through the upper half of an aecium. At the lower end of the drawing (*f-g*) is the edge of the looser, space-making tissue, and above this area are the sporogenous cells. All through this upper region are cells with two or three nuclei (pl. 14, B, *a, b, c, d, e, f*), no one of which looks as though it were the immediate product of fusion. The shape and sequence of these cells suggest that sporophytic hyphae have grown in from some point of origin above the aecium.

Plate 14, C, shows the upper central part of a slightly older aecium of a 12-day infection, in which the evidence is even stronger of an invasion from above. Apparently a mixture of diploid and haploid hyphae entered at *a*, grew downward, branching freely, and then spread to right and left. With the exception of the cell at *b*, none of the cells appears to be the direct result of fusion.

In many cases, however, fusions do continue to occur in the lower part of the sporogenous area. In Plate 14, A, the cells at *a, b*, and *c*, indicate by their shape that two cells have combined to produce them, although here, as in some of the other cases cited, there are binucleate cells in the mycelium outside the aecium. Plate 15, A, *d* and *e*, shows other cells from the sporogenous area whose shape indicates fusion.

At this time binucleate cells in the aecium are rapidly increasing in numbers, trinucleate cells are less common, and cells with more than three nuclei are exceptional. Hyphae with different nuclear content in successive cells may still be found. In Plate 12, E, the sequence is 2-2-3; in Plate 14, C, *f*, it is 1-2-1; and in Plate 15, A, *b*, it is 4-2-3.

The haploid, unisexual aecium, if left to itself (pls. 8, 9, 10), develops to a certain stage, becomes differentiated into areas, forms irregular multinucleate cells in what corresponds to the sporogenous area, then deteriorates and dies. In the earlier stages of this development an invasion of sporophytic hyphae or perhaps even of haploid hyphae of opposite sex may become incorporated in its growth and change it to a normal fertile aecium.

Just how late in development such an invasion can become effective is not certain. One aecium had reached the earlier signs of deterioration. The cells of the sporogenous area were loosely spaced and had become less dense in content. The first irregular multinucleate cells (see pl. 12, J) had developed into typical forms. But several sporophytic hyphae were found extending into this aecium along its upper border, and near to these occurred the cell shown in Plate 12, I, which strongly suggests fusion between a slender invading hypha and a local cell. This aecium might be able to develop spores in normal fashion.

Spore formation begins about the twelfth day or, more rarely, as early as the tenth day. Plate 14, C, shows the layer of basal cells forming along the line from *c* to *e*. Thick heavy cells are beginning to grow down in more or less parallel alignment at the boundary of the space-making tissue. The first spore mother cell has been cut off at *d*.

A slightly more advanced stage (pl. 15, B) shows a part of the sporogenous area of a 12-day infection and the edge of the space-making tissue, (*d-e*). Sporophytic cells throughout the sporogenous area are growing downward toward the space-making tissue. Some of these (pl. 15, B, *b, f*) start at the boundary between the two areas and push out into the looser tissue below. Others higher up (*a, c, g, h*) grow downward and will eventually come into line with the former.

A majority of the sporophytic cells are now binucleate, but a few (pl. 15, B, *b*; D, *f*; E, *b*, *f*; C, *b*) have 3, 4, or even 5 nuclei.

This growth downward of a sporophytic cell may begin at one end or at any point along its length, probably varying according to the available space for such growth. Plate 15, E, *a* and *b*, and Plate 16, B, *d*, show the end of the cell pushing down. In Plate 15, B, *b*, the best opportunity for growth is near one end. Plate 15, B, *h*, shows the downward growth arising as a median branch, giving to the hypha a 3-prong shape. Plate 14, B, *h*, and Plate 15, D, *a-b*, show other triangular cells, which will give rise to the "2-legged" cells frequently figured in other rusts and shown in Plate 16, A, *a*.

Ordinarily, in fertile aecia making definite progress toward spore formation, the cells with more than one nucleus do not die. Plate 15, C, shows (in lower magnification than pl. 15, B) a detail from a sporogenous area, with several dead cells suggestive of those found in sterile infections, in a 14-day infection, the other aecia of which have formed young spores.

Plate 15, D, shows another detail from a sporogenous area. Some of the upper sporophytic cells, as at *c*, are still inactive; others (*d*) are beginning to grow down; and one (*e*) has formed the first spore mother cell. At *f* is a trinucleate cell with the scant cytoplasm characteristic of the space-making tissues.

Plate 15, E, already referred to, shows still other details. Some of the sporophytic cells (*i*) have not started to push down, others (*a*, *b*), have just started, while still others (*c*, *d*) have a fully formed basal cell, which has formed the first spore mother cell. In one of these, both the basal cell (*f*) and the first spore mother cell (*e*) are trinucleate.

When basal cells begin to carry on nuclear and cell divisions, their protoplasm concentrates in the lower end of the cell, leaving the upper end vacuolate. In a young sporogenous area all the cells have dense contents. By the time the sporophytic growth dominates the area the remaining gametophytic cells are clear and almost empty (pl. 16, B, *c*, *f*), contrasting sharply with the binucleate cells of the same region.

Mention has been made of what appear to be cell fusions in the sporogenous area of the aecium (pl. 12, C, *d*; I; pl. 14, A, *a*, *b*, *c*; C, *b*; pl. 15, A, *d*, *e*) before the onset of spore formation. Even in the later stages of development fusions can be found. In Plate 15, A, *c*, appearances indicate that two young basal cells have fused; the combination cell has six nuclei of different sizes. In Plate 16, B, *e*, two basal cells joined; apparently each contained at least two nuclei at the time of joining. In Plate 16, A, *b*, *c*, and *d*, three young spore mother cells of adjoining spore chains have fused; the combination cell has eight nuclei of different sizes. It is evident that

EXPLANATORY LEGEND FOR PLATE 15

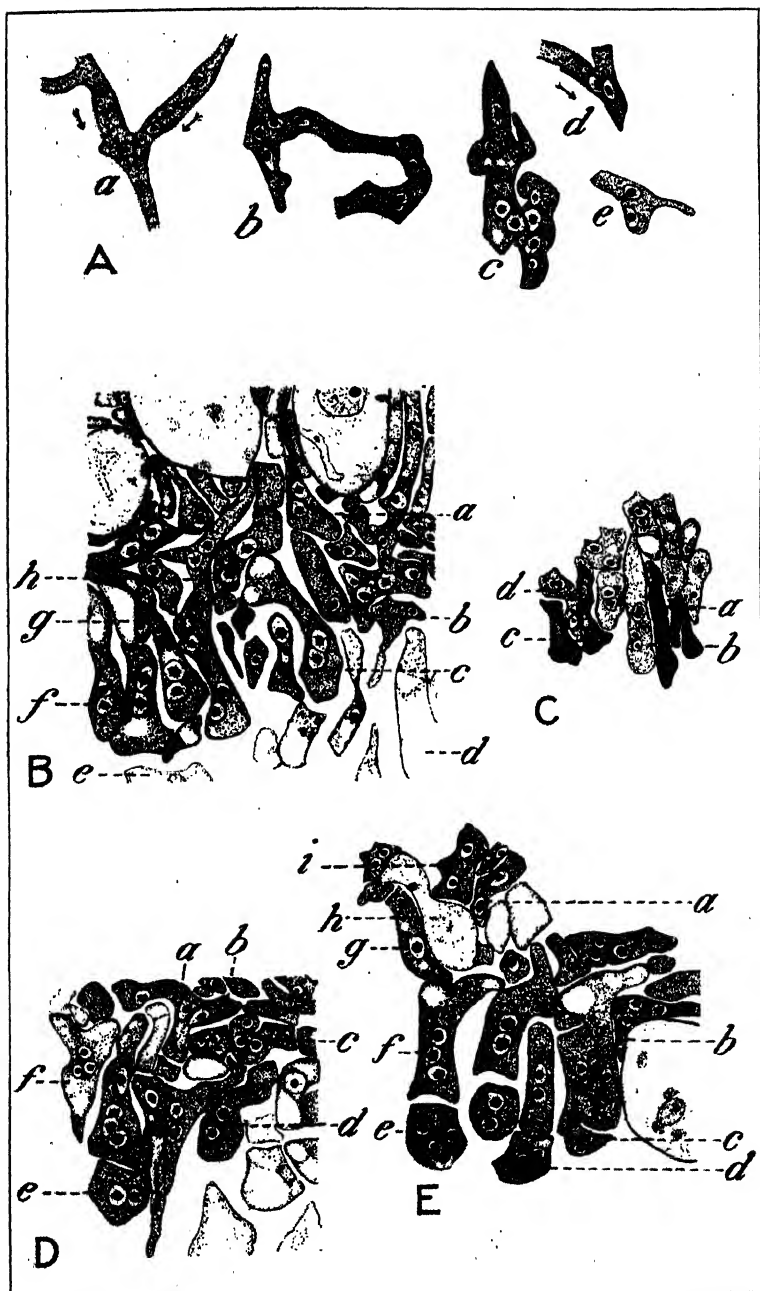
A.—Details from 12-day fertile infection: *a*, Possible hyphal fusion; *b*, hypha from sporogenous area, with quadrinucleate, binucleate, and trinucleate cells; *c*, fusion of basal cells; *d* and *e*, probable cell fusions in sporogenous area. Arrows indicate direction of growth. $\times 1,020$.

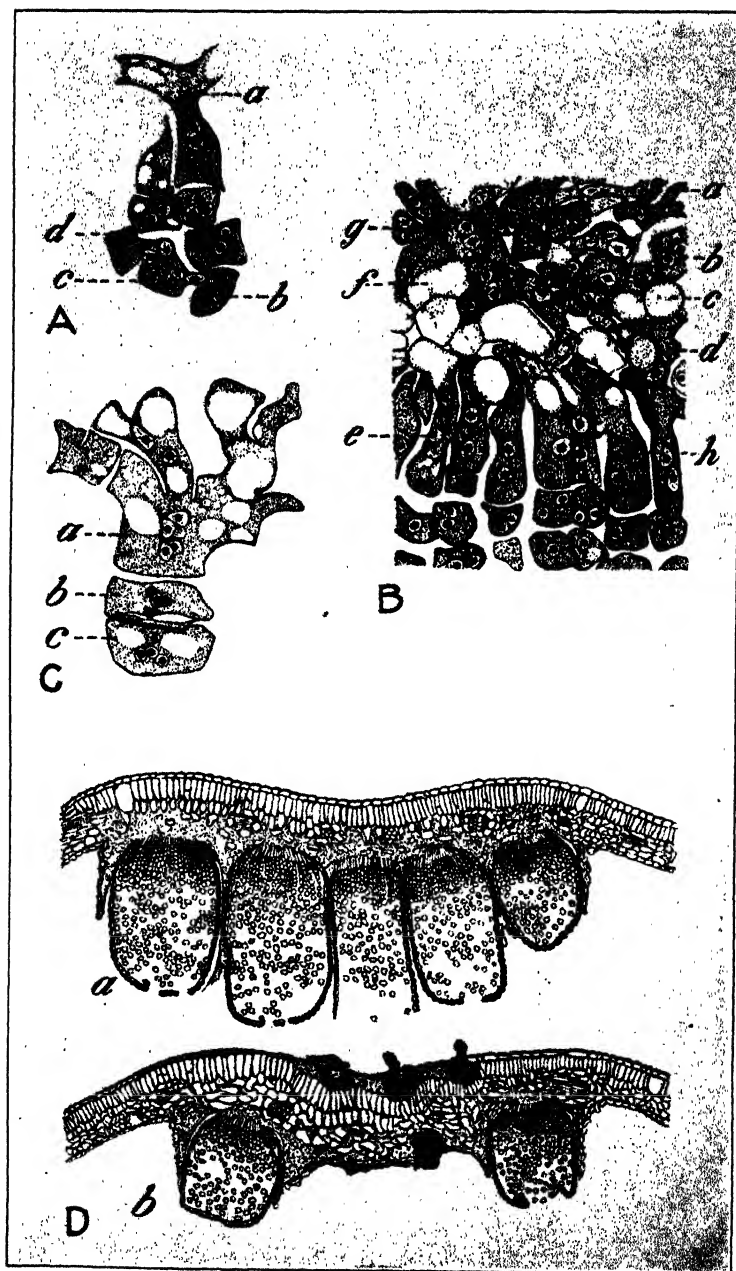
B.—Portion of sporogenous area of a 12-day fertile infection: *a*, *b*, *c*, *f*, *g*, and *h*, Basal cells forming; *d* to *e*, border of space-making tissue. $\times 1,020$.

C.—Detail of sporogenous area of 14-day fertile infection: *a* and *c*, Dead basal cells; *b* and *d*, living basal cells. $\times 040$.

D.—Detail of sporogenous area of 12-day fertile infection: *a*, *b*, *c*, *d*, and *f*, Basal cells forming; *e*, spore mother cell. $\times 1,020$.

E.—As in D: Younger basal cells starting at *a* and *b*; fully formed basal cells and first spore mother cells below at *c*, *d*, *e*, and *f*; *g*, binucleate cell, one at *i* not yet started; *h*, dying haploid cell. $\times 1,020$.





FOR EXPLANATORY LEGEND SEE OPPOSITE PAGE

the presence of two or more nuclei in a cell does not always prevent further fusions. Plate 16, C, shows evidence of this; a giant basal cell (*a*) has formed, evidently by multiple fusion, and is giving rise to a giant spore chain (*b, c*), of quadrinucleate cells. These irregularities are exceptional. The great majority of basal cells are binucleate, act as independent units, and produce chains of binucleate spores.

Under favorable conditions there are open aecia by the eighteenth day. If the infection has not been cramped by the crowding together of several infections nor deformed by encountering a large vein, it is circular in outline. The group of spermogonia or the denuded area formerly occupied by spermogonia is at the center of the circle on the upper surface. There is a similar, but smaller, spermogonial area at the lower surface. Opening on the lower surface are the aecia. Plate 16, D, shows two sections through a small 18-day infection. A central section (pl. 16, D, *b*) shows spermogonia (no longer functioning) at the center, flanked on either side by aecia. A section nearer the margin (pl. 16, D, *a*) shows a continuous row of aecia.

DISCUSSION

Although *Puccinia coronata*, *P. graminis*, and *P. triticina* (1, 2) differ in many respects, the entry of the sporidium into the epidermal cell and the formation of the primary hypha and its mode of branching are very similar in the three species.

The first difference appears when branches from the primary hypha penetrate into the subepidermal region. In *Puccinia graminis* and *P. triticina* these hyphae grow downward at once into the spongy mesophyll and feed and spread there for several days; then a new growth of hyphae returns to the subepidermal region for the formation of spermogonia. In *P. coronata* the dominant growth for the first week is subepidermal. The hyphae force a passageway between epidermis and palisade and spread, forming a more or less circular stroma on which the spermogonia develop. From any point in this mat, hyphae may grow downward into the mesophyll.

This formation of a subepidermal web of hyphae between epidermis and palisade depends upon the ability of *Puccinia coronata* to split apart the host cell walls and force a passage between them. The absence of a natural intercellular space is not an insurmountable obstacle, in thin-walled tissue at least, which, even in the smaller veins, is traversed by hyphae.

The aecia in a sterile infection of *Puccinia coronata* regularly form multinucleate cells, although the infection as a whole must be regarded as gametophytic. Bensaúde (4) and Sass (24), in studies of agarics, record the regular occurrence of a multinucleate phase in the young

EXPLANATORY LEGEND FOR PLATE 16

A.—Detail of spore chains from a 12-day fertile infection: *a*, 2-logged cell; *b, c*, and *d*, fusion of three spores. $\times 1,020$.

B.—Detail from sporogenous area of 12-day fertile infection: *a, b*, and *g*, Dense sporophytic cells above; *c* and *f*, nearly empty gametophytic cells; *d*, new basal cell forming; *e*, fusion between basal cells; *h*, basal cells with young spore chains. $\times 1,020$.

C.—Twelve-day fertile infection: *a*, Giant basal cell formed probably by multiple fusion, giving rise to a spore chain of giant quadrinucleate cells (*b, c*). $\times 1,020$.

D.—Semidiagrammatic sections through an 18-day fertile infection: *a*, Lateral section showing a row of aecia; *b*, median section showing the patch of dead spermogonia at the center flanked by open living aecia. $\times 45$.

primary or gametophytic mycelium. Lindfors (18) found that in *Trachyspora alchimilliae* (Pers.) Fekl. the uninucleate mycelium of the primary uredo generation may become multinucleate in the small intercellular spaces of the embryonic tissues of the host, but becomes uninucleate again as those tissues expand. All these instances of multinucleate cells in the haploid generation occur in vegetative mycelium; they are therefore not directly comparable to the present case.

It is not known whether multinucleate cells similar to those of *Puccinia coronata* are to be found in unisexual aecia of other species of rusts. Until the announcement by Craigie (11), in 1927, of heterothallism in the rusts, it was not even known that unisexual infections existed. There are many references in the earlier literature of the rusts to the presence of multinucleate cells in aecia, and they are sometimes spoken of as "pathological." Olive (23) finds "a multinucleated stage" in a large proportion of 50 species examined. It would be interesting to know whether some of these occurred in sterile aecia not recognized as such.

In *Puccinia coronata* the multinucleate cells in the sterile aecium form at the time and in the place where spore-forming activities would be going on in a fertile aecium; their formation seems to be a futile expression of a reproductive tendency. The sporogenous area in a fertile aecium is richly supplied with food. A sterile aecium is similarly organized, and possibly the nutriment supplied to this area parallels, for a time at least, the supply that would have been needed for spore formation. Apparently, cells in this area in the unisexual aecium can grow and carry out the rapid nuclear divisions that would accompany spore formation, but can not form the spores. The resulting multinucleate cells become badly overgrown and irregular in shape and sooner or later deteriorate and die.

There is some evidence in the literature on rusts that development does not always stop at this point in the unisexual aecium. Moreau (19, 20) reported the widespread occurrence of a uninucleate variety of *Endophyllum euphorbiae* (DC.) Wint. Mycelium, aecia, and aeciospores were haploid throughout. Haploid aecia with regular spore chains of uninucleate aeciospores have been found by Kursanov (17) in three different collections of *Aecidium punctatum* Pers. The aecia fully resembled sporophytic aecia in all morphological details. The later history of these uninucleate aeciospores is not known. Kursanov (17) also found uninucleate aeciospores in *A. leucospermum* DC. These spores were capable of germination, but no infections were obtained. Dodge (14) found in the short-cycle rust *Caeoma nitens* Schw. a strain without spermogonia or with only vestigial spermogonia. There were no cell fusions at the base of the aecia; the spores were uninucleate and germinated by producing 2-cell promycelia with two sporidia.

The foregoing data are open to several interpretations. One possibility is that, when fertilization fails to take place, the unisexual aecium stops at different points in its development in different rusts. In *Puccinia graminis* and *P. triticina* the haploid aecium forms and the sporogenous area takes shape, but with rare exceptions the cells of the latter remain uninucleate and soon become impoverished and die. In *P. coronata* the sporogenous area of the unisexual aecium lives longer and its cells may carry out several nuclear divisions, but ultimately it dies without forming spores. In *Endophyllum euphor-*

biae, *Aecidium punctatum*, *A. leucospermum*, and *Caeoma nitens*, the haploid aecium can develop to the spore-forming stage and produce haploid spores.

Andrus (3) believes the hyphae that emerge upon the surface of the leaf are trichogynes, growing directly from "eggs" in the sporogenous layer of young aecia. He says (3, p. 567):

At an early stage previous to fertilization, when the aecial primordia are in process of formation, that is, 8 to 12 days after inoculation, no hyphae are found in stomata or protruding between epidermal cells.

In *Puccinia coronata*, on the contrary, a mycelium so young that it has not yet reached the lower epidermis of the leaf and sometimes has not even started to form spermogonia may nevertheless have formed several emergent hyphae (pl. 6, B and C) growing up between epidermal cells and along under the cuticle. These can not have grown from aecia, since aecial primordia do not start to form until two or three days later.

In *Puccinia coronata*, hyphae may emerge upon the surface of the leaf at any point in the infected area after the fourth or fifth day of development. They form at the upper surface first and at the lower surface later, when the parasite becomes established there. A receptive hypha may grow out from vegetative mycelium, or it may start as a branch from a young paraphysis in a spermogonium or from any point in the subepidermal web of hyphae, or it may grow from a young aecium to a stoma adjoining it. Relatively few of these receptive hyphae can be traced directly to an aecium. The emergent hyphae function as trichogynes in receiving the spermatial nuclei, but they show no further similarity to the trichogynes described in other fungi and in algae.

It should be pointed out, however, that in a monosporidial infection all the hyphae belong to one connected system. In that sense an emergent hypha, no matter how remote it may be from an aecium, is still connected with it, after aecia begin to form, by a series of hyphae. If the process of "progressive diploidization" described by Buller (7) should be proved to exist in rusts, it would explain how nuclei received at any point in the mycelium could be transmitted by successive nuclear divisions and migrations to any other point. The route to an aecium might be fairly direct or might involve irregular detours, but it would be feasible, for the distances involved are small.

In *Puccinia graminis* (1), *P. triticea* (2), and *P. coronata*, it has been repeatedly observed that when the sporophyte generation is spreading from its point or points of origin mycelial cells may contain from one to five nuclei, an occasional cell may have no nucleus, the nuclei may be of different sizes and show different arrangements within the cell, septa are often ill-defined, and successive cells in a hypha often do not have the same number of nuclei. In the systemic infections of *P. suaveolens* Rostr., in the neighborhood of the spermogonia and the primary uredo, Kursanov (17) found a mixture of gametophytic and sporophytic hyphae. In the latter the number of nuclei in successive cells of the same hypha varied. He reports the following sequences: 3-1-2; 2-2-2-3-2-2-1; 2-3-3-3-2-2; 2-2-1; and 3-2-1.

This seeming chaos is difficult to account for on the basis of the regular apical growth of diploid hyphae, but if the surplus nuclei in

the cells of the sporophytic hyphae are transient occupants, on their way in the process of diploidizing ready-formed haploid mycelium, the apparent disorderliness takes on new meaning. On this basis, too, an explanation suggests itself for the seemingly casual cell fusions found at different stages in the development of the aecium, in some cases between cells already containing more than one nucleus. A sporogenous area, established in part by a rather plastic and variable system of nuclear migrations, would be apt to contain some unpaired nuclei. It is conceivable (but, of course, not proved) that in these last-minute cell fusions an interchange of nuclei takes place by which such nuclear imbalances are corrected.

Evidence is increasing that the sporophyte generation originates outside the aecium in some species, but how widespread in the Uredineae this will be found to be is a matter for conjecture only. The occasional 2-legged basal cell, so long regarded as positive proof of the origin of the sporophyte in the aecium, is open, in some cases at least, to other interpretations. Moreover, it is evident from studies of *Puccinia coronata* that the presence of an occasional supplementary cell fusion in the aecium prior to the formation of the basal cells does not exclude the possibility of earlier sporophytic mycelium outside the aecium. Detailed investigation of a large series of rusts is necessary before any generalizations can be attempted on this subject.

Bisexual mycelium may originate at the leaf surface where emergent hyphae meet spermatia, but the evidence is still scant that it can originate also within the leaf by hyphal fusions where two mycelia interlace. Even if hyphal fusions were common, they would be difficult to find; but it is probable that they do take place.

The sporophyte generation is known to originate by means of hyphal fusions in other groups of fungi. Bensaúde (4) found that in *Coprinus* the dicaryophyte begins with the fusion of a (+) and a (-) mycelial cell or of a mycelial cell and an oidium. This has since been found to be general in heterothallic hymenomycetes. A few cases of hyphal fusion are known in the ascomycetes. Gwynne-Vaughan and Williamson (16) found that in *Humaria granulata* Quel. all single-spore mycelia produce well-formed multinucleate oogonia, which, however, remain sterile and ultimately degenerate. No antheridia have been found. Although all the individuals are morphologically female, they may nevertheless be divided into 2 clean-cut groups, 1 (+) and 1 (-). When a (+) mycelium meets a (-) mycelium, hyphal fusions take place initiating bisexual mycelium, on which are formed fertile oogonia that produce ascogenous hyphae and asci.

In other ascomycetes fertilization in the oogonium has been questioned. Even the famous *Pyronema confluens* (Pers.) Tul. is questioned, for Moreau and Moreau (21) maintain that the nuclei of the antheridium and the trichogyne degenerate and that oogonial nuclei issue two by two to form the ascogenous hyphae. They state (21, p. 91) that "Ni chez les Champignons des Lichens, ni chez le *Pyronema*, ni chez le *Sphaerotheca*, ni chez le *Polystigma*, nous n'avons observé de fécondation à l'origine du périthèce," and believe in (21, p. 93) "l'absence de toute fécondation actuelle à la base des périthèces des Ascomycètes."

They admit the possibility in heterothallic forms, however, of hyphal fusions between (+) and (-) mycelia, by which nuclei of

the two sexes could be brought together. One may not accept these generalizations, but it is evident that there is increasing reason for belief that the origin of the sporophyte generation by hyphal fusions is fairly common in the higher fungi.

In *Puccinia coronata* the basal cells just ready to start spore chains are predominantly binucleate but may contain as many as four or five nuclei. This multinucleate condition was more common and more pronounced in *P. graminis* and *P. triticea*, in which young basal cells often contained 5 or 6 nuclei and occasionally 8 or 10. Similar precocious nuclear divisions were found by Colley (10) in basal cells of *Cronartium ribicola* Fisch. In the fertile aecium these prematurely formed nuclei are utilized later in spore formation. In the sterile aecium, in *P. coronata*, the nuclei merely accumulate in monstrous cells that then die.

SUMMARY

The sporidium of *Puccinia coronata* on a leaf of *Rhamnus cathartica* pushes out a short beak, which becomes attached to the epidermal cell, pierces the wall, and grows into the cell. The sporidial nucleus divides before entry. A primary hypha of four to six cells is formed in the epidermal cell. From each cell, in turn, a branch grows down into the subepidermal region.

For the first week the dominant growth of the rust is subepidermal, the hyphae separating the epidermis from the palisade and forming a web of hyphae between the two layers. This web spreads radially beneath the epidermis. From it hyphae grow down into the mesophyll. Later, a smaller stroma may form next the lower epidermis.

Each spermogonium begins as a small circular patch on the subepidermal stroma from which hyphae grow upward. Heavy upright cells at the center of this group serve as buffer cells, pushing up the epidermis. From the margin of the patch a circle of slender paraphyses curve upward and inward, the tips meet, then grow out together and pierce the epidermis forming the ostiole. Numerous slender spermatophores grow in from the sides and base of the spermogonium and set free spermatia into the central cavity. Spermogonial exudate is not abundant, and spermogonia are not long-lived. At the same time hyphae near either leaf surface grow out to the surface, pushing into stomatal apertures, or between epidermal cells or even through an epidermal cell. These hyphae, which may come from vegetative mycelium, from a spermogonium, or from a young aecium, serve to receive spermatial nuclei.

Puccinia coronata is heterothallic. The unisexual infection, if carefully isolated, produces spermatia but no aeciospores. Aecia form but remain sterile.

The sterile aecium begins as a little nest of uninucleate cells near the lower epidermis. It grows and becomes differentiated into an upper half of dense cells and a lower half of looser cells. In the upper half are cells containing more than one nucleus. These cells grow and their nuclei divide, sometimes several times, but sooner or later they deteriorate and die.

When spermatia of opposite sex are transferred to the surface of an infected area, their nuclei enter the exposed tips of the receptive hyphae, initiating there the sporophytic generation. From these

points the sporophyte spreads by growth of diploid hyphae, probably also by nuclear divisions and migrations through ready-formed haploid hyphae.

The aecium that is to form spores may contain both sporophytic and gametophytic hyphae from the start, or may begin as a haploid, unisexual aecium and be invaded later by the sporophytic component. An occasional cell fusion is found in the fertile aecium, sometimes between uninucleate cells, sometimes between cells one or both of which contain more than one nucleus. Basal cells are predominantly binucleate, occasionally multinucleate. With but few exceptions the spores are binucleate.

LITERATURE CITED

- (1) ALLEN, R. F.
1930. A CYTOLOGICAL STUDY OF HETEROTHALLISM IN PUCCINIA GRAMINIS. Jour. Agr. Research 40:585-614, illus.
- (2) ———
1931. A CYTOLOGICAL STUDY OF HETEROTHALLISM IN PUCCINIA TRITICINA. Jour. Agr. Research 44:733-754, illus.
- (3) ANDRUS, C. F.
1931. THE MECHANISM OF SEX IN UROMYCES APPENDICULATUS AND U. VIGNAE. Jour. Agr. Research 42:559-587, illus.
- (4) BENSÂUDE, M.
1918. RECHERCHES SUR LE CYCLE ÉVOLUTIF ET LA SEXUALITÉ CHEZ LES BASIDIOMYCÈTES. 156 p., illus. Nemours.
- (5) BLACKMAN, V. H.
1904. ON THE FERTILIZATION, ALTERNATION OF GENERATIONS, AND GENERAL CYTOLOGY OF THE UREDINEAE. Ann. Bot. [London] 18:[323]-373, illus.
- (6) ——— and FRASER, H. C. I.
1906. FURTHER STUDIES ON THE SEXUALITY OF THE UREDINEAE. Ann. Bot. [London] 20:[35]-48, illus.
- (7) BULLER, A. H. R.
1930. THE BIOLOGICAL SIGNIFICANCE OF CONJUGATE NUCLEI IN COPRINUS LAGOPUS AND OTHER HYMENOMYCETES. Nature [London] 126:686-689, illus.
- (8) CHRISTMAN, A. H.
1905. SEXUAL REPRODUCTION IN THE RUSTS. Bot. Gaz. 39:267-275, illus.
- (9) ———
1907. THE NATURE AND DEVELOPMENT OF THE PRIMARY UREDOSPORE. Wis. Acad. Sci., Arts, and Letters, Trans. 15:[517]-526, illus.
- (10) COLLEY, R. H.
1918. PARASITISM, MORPHOLOGY, AND CYTOLOGY OF CRONARTIUM RIBICOLA. Jour. Agr. Research 15:619-660, illus.
- (11) CRAIGIE, J. H.
1927. EXPERIMENTS ON SEX IN RUST FUNGI. Nature [London] 120:116-117, illus.
- (12) ———
1927. DISCOVERY OF THE FUNCTION OF THE PYCNIA OF THE RUST FUNGI. Nature [London] 120:765-767.
- (13) ———
1928. ON THE OCCURRENCE OF PYCNIA AND AECIA IN CERTAIN RUST FUNGI. Phytopathology 18:1005-1015, illus.
- (14) DODGE, B. O.
1924. UNINUCLEATED AECIDIOSPORES IN CAEOMA NITENS AND ASSOCIATED PHENOMENA. Jour. Agr. Research 28:1045-1058, illus.
- (15) GWYNNE-VAUGHAN, H. C. I., and WILLIAMSON, H. S.
1930. CONTRIBUTIONS TO THE STUDY OF HUMARIA GRANULATA QUEL. Ann. Bot. [London] 44:127-145, illus.
- (16) HANNA, W. F.
1929. NUCLEAR ASSOCIATION IN THE AECIUM OF PUCCINIA GRAMINIS. Nature [London] 124:267.

- (17) KURSANOV, L.
1922. RECHERCHES MORPHOLOGIQUES ET CYTOLOGIQUES SUR LES URÉDINÉES. Bul. Soc. Nat. Moscou (n. s.) 31:1-129, illus.
- (18) LINDFORS, T.
1924. STUDIEN ÜBER DEN ENTWICKLUNGSVERLAUF BEI EINIGEN ROSTPILZEN AUS ZYTOLOGISCHEN UND ANATOMISCHEN GESICHTSPUNKTEN. Svensk Bot. Tidskr. 18:1-84, illus.
- (19) MOREAU, Mme. F.
1911. SUR L'EXISTENCE D'UNE FORME ÉCIDIENNE UNINUCLÉÉE. Bul. Soc. Mycol. France 27:[489]-493, illus.
- (20) ———
1915. NOTE SUR LA VARIÉTÉ UNINUCLÉÉE DE L'ENDOPHYLLUM EUPHORBIAE (DC.) WINTER. Bul. Soc. Mycol. France 31:[68]-70, illus.
- (21) MOREAU, F., and MOREAU, Mme. F.
1930. LE DÉVELOPPEMENT DU PÉRITHÈCE CHEZ QUELQUES ASCOMYCÈTES. Rev. Gén. Bot. 42:65-98, illus.
- (22) NEWTON, M., JOHNSON, T., and BROWN, A. M.
1930. A PRELIMINARY STUDY ON THE HYBRIDIZATION OF PHYSIOLOGIC FORMS OF PUCCINIA GRAMINIS TRITICI. Sci. Agr. 10:721-731, illus.
- (23) OLIVE, E. W.
1910. THE PRESENT STATUS OF THE CYTOLOGY OF THE RUSTS. Science (n. s.) 31:437-438.
- (24) SASS, J. E.
1929. THE CYTOLOGICAL BASIS FOR HOMOTHALLISM AND HETEROTHALLISM IN THE AGARICACEAE. Amer. Jour. Bot. 16:663-701, illus.
- (25) STAKMAN, E. C., LEVINE, M. N., and COTTER, R. U.
1930. ORIGIN OF PHYSIOLOGIC FORMS OF PUCCINIA GRAMINIS THROUGH HYBRIDIZATION AND MUTATION. Sci. Agr. 10:707-720.
- (26) WATERHOUSE, W. L.
1929. A PRELIMINARY ACCOUNT OF THE ORIGIN OF TWO NEW AUSTRALIAN PHYSIOLOGIC FORMS OF PUCCINIA GRAMINIS TRITICI. Linn. Soc. N. S. Wales, Proc. 54:96-106, illus.

SOME PHYSIOLOGICAL STUDIES OF POTATOES IN STORAGE¹

By R. C. WRIGHT

Physiologist, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture

INTRODUCTION

The purpose of the investigation begun in 1924 and described herein was to determine the changes in sugar content, the rate of respiration, and the intercellular or internal atmospheric composition of potatoes stored at different temperatures and sampled at succeeding periods during storage. Studies along these lines were carried on simultaneously, or as nearly so as practicable. Investigations were made also on changes in potatoes that had been held in storage at 32° F. for a given time and had then been transferred and held at several different higher temperatures, and in potatoes that had been frozen.

MATERIAL USED

The potatoes used in these investigations were Irish Cobbler, Green Mountain, and Triumph varieties true to name and grown by the United States Department of Agriculture at Presque Isle, Me. They were well matured, carefully selected for uniformity of size, and were free from evidence of disease. In 1924 the varieties were harvested on September 24 and stored in crates in a potato storage house at a temperature of about 50° F. until shipped. They were shipped in barrels by freight directly to Washington, D. C., and arrived at the laboratory on November 1.

On arrival at Washington all the potatoes of each variety were carefully emptied on the floor in such a way as to mix together the contents of the barrels. Potatoes were then selected from different locations in the pile in order to secure composite samples as nearly comparable as possible. In handling the potatoes care was taken to avoid skinning or other mechanical injury that might cause physiological disturbance. As the potatoes were well matured and the skins rather firm, practically no skinning occurred.

CHANGES IN POTATOES DURING CONTINUOUS STORAGE AT DIFFERENT CONSTANT TEMPERATURES

On November 5 lots of each of the three varieties were put in storage at controlled temperatures of 32°, 36°, 40°, 50°, 60°, and 70° F. and allowed to remain continuously for periodic sampling. The relative humidity was maintained at approximately 85 per cent in order to prevent undue loss of moisture.

¹ Received for publication Feb. 18, 1932; issued November, 1932.

SUGAR CONTENT

In order to determine the initial sugar content for each variety, representative composite samples were taken for analysis at the beginning of the experiment. Following this, samples were taken periodically, on November 26, December 17, February 5, March 24, and June 1.

Cylindrical plugs about 15 mm in diameter were taken from each of about 12 representative potatoes by cutting with a cork borer from side to side through the center. The plugs from each lot were put through a sampling press that reduced the material to a fine pulp, which was then mixed by thorough stirring. Duplicate 50 g portions of each sample were weighed out and covered with approximately 85 per cent alcohol in 400 c c beakers and boiled for a few moments. The samples were then washed into 500 c c volumetric flasks, brought to volume, and allowed to stand, with occasional shaking, until all the samples had been taken.

After filtering, a 50 c c aliquot part of the alcoholic extract was evaporated in order to remove the alcohol. It was then brought to nearly 250 c c with water; 5 c c of neutral lead acetate was added for clearing; and the mixture was then brought to exact volume, filtered, and delead with sodium oxalate. Reducing sugar was determined in a 50 c c aliquot portion by using Bertrand's modification of the Munson and Walker method. To another 50 c c aliquot portion of the clarified and delead solution 5 c c of hydrochloric acid was added, and the whole brought to 100 c c. After inversion in the cold for 24 hours and then neutralizing, total sugar was determined on a 50 c c aliquot by the method previously used to determine the reducing sugar. Sucrose was then determined by difference. The averages, calculated on the basis of the weight of the fresh tissue and expressed as percentage of dextrose, are shown in Table 1 and in Figure 1.

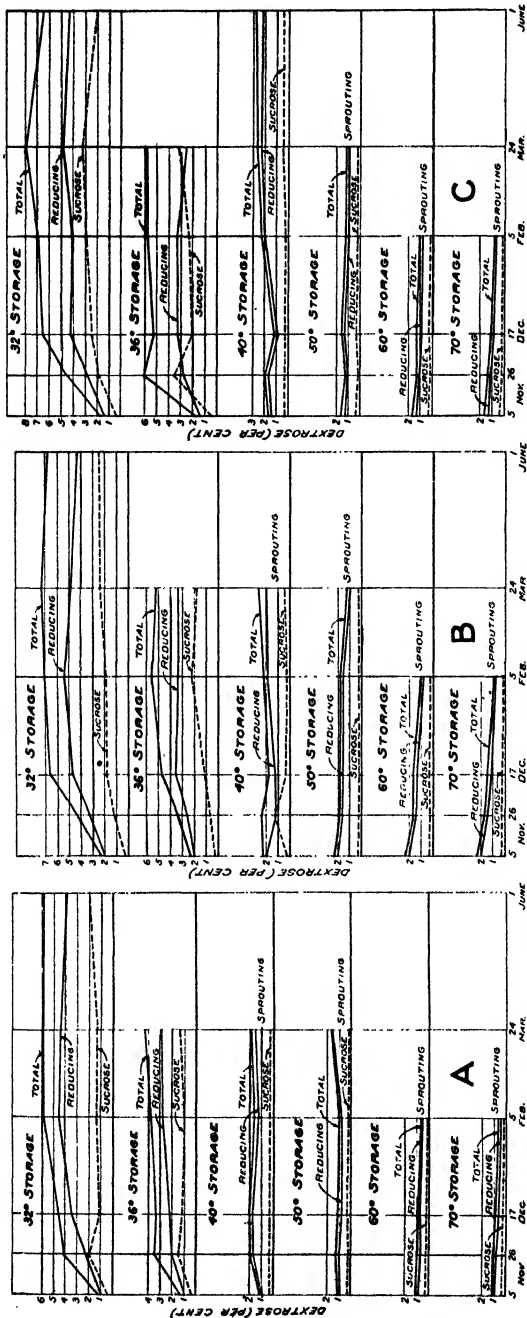


FIGURE 1.—Progressive changes in the sugar content of Irish Cobbler (A), Green Mountain (B), and Triumph (C) potatoes at various storage temperatures, degrees Fahrenheit

In all three varieties stored at 32° and 36° F., disregarding certain fluctuations, a general progressive increase in reducing sugar, sucrose, and total sugar was found at each sampling, including samples taken on March 24. This was followed by a tendency to decrease, as shown on June 1. The greatest amount of total sugar accumulated was at 32°. The sugar content of the Irish Cobbler increased from 1.18 to 5.87 per cent, Green Mountain from 2.26 to 7.28 per cent, and Triumph from 1.65 to 7.97 per cent, or percentage increases of 397, 222, and 383, respectively. (Table 1.) The most rapid increase in sugar occurred between the beginning of the experiment and the first sampling date, that is, during the first 21 days of storage. In potatoes stored at 40° there was apparently a slight general increase in sugar, but the quantity seemed to fluctuate from one sampling date to another. This fluctuation may have been due to analytical error or variation in sampling, although great care was taken at each sampling to select representative tubers from different locations in the baskets. With the exception of the Irish Cobbler variety, potatoes stored at 50° showed a general decrease in sugar on March 24. In this variety more sugar was found than at the beginning, but this is considered to be due to some experimental error. At 60° and 70° the total-sugar content in general decreased during storage to one-half or less. With certain exceptions, the tendency was for a loss of sugar, increasing in proportion as the temperature increased from 50° to 70°.

The relatively high sugar content of potatoes stored at 32° and at 36° F. explains much of the unpalatability due to excessive sweetness commonly recognized in tubers stored at low temperatures. Sweetness was readily detected by taste in uncooked specimens stored at these low temperatures, as compared with those stored at 40° or above.

RESPIRATION

In the same storage lots of Irish Cobbler and Green Mountain potatoes on which sugar analyses were made periodically, the relative rates of respiration, expressed as milligrams of carbon dioxide given off per kilogram of material per hour, were determined on three dates during the storage period. The method and apparatus used were similar to those described by Magness and Diehl (8).² The respiration tests were carried on in duplicate on approximately 3-kg samples. The first test was begun on November 7, two days after the potatoes were placed in storage; similar tests were begun on January 17 and on March 24. The duration of each test was between 300 and 400 hours. After each test the tubers used were discarded, and new groups of tubers were selected for each succeeding test. The results obtained at the storage temperatures indicated are presented in Table 2. The respiration rates, as determined in continuous runs of approximately 24 hours each throughout these tests, proved to be fairly uniform; for the sake of brevity only averages for entire test periods are given herein.

² Reference is made by number (italic) to Literature Cited, p. 555.

TABLE 2.—Seasonal respiration of Irish Cobbler and Green Mountain potatoes in storage at various temperatures

IRISH COBBLER					
Test begun	Storage temperature	CO ₂ per kilogram per hour	Duration of test	Weight of sample	
				Beginning	End
	° F.	Mg	Hours	Kg	Kg
Nov. 7.....	32	6.22	408	3.610	3.592
	36	9.28	380	3.622	3.510
	40	8.48	404	3.800	3.792
	50	5.92	387	2.675	2.661
	60	8.75	263	3.682	3.667
Jan. 17.....	70	8.91	405	3.575	3.549
	32	4.25	332	2.607	2.603
	36	4.66	288	2.651	2.636
	40	3.92	339	2.423	2.423
	50	4.35	338	2.514	2.514
Mar. 24.....	60	10.40	389	2.828	2.818
	70	12.42	285	2.993	2.962
	32	3.27	402	3.064	3.060
	36	4.52	405	3.274	3.272
	40	3.68	386	2.975	2.972
	50	4.50	388	2.415	2.411
	60	(a)	(a)	(a)	(a)
	70	(a)	(a)	(a)	(a)
GREEN MOUNTAIN					
Nov. 7.....	32	5.85	408	3.512	3.502
	36	7.07	380	3.422	3.415
	40	5.11	404	3.660	3.651
	50	6.40	387	2.641	2.631
	60	8.63	386	3.650	3.632
Jan. 17.....	70	10.06	405	3.645	3.621
	32	4.20	332	2.508	2.508
	36	3.94	288	2.843	2.831
	40	3.57	339	2.752	2.752
	50	3.73	338	2.177	2.177
Mar. 24.....	60	5.80	289	2.855	2.845
	70	9.38	285	2.885	2.856
	32	2.99	402	2.997	2.994
	36	3.65	309	3.425	3.416
	40	2.93	386	3.422	3.420
	50	4.66	388	2.037	2.030
	60	(a)	(a)	(a)	(a)
	70	(a)	(a)	(a)	(a)

* Sprouting.

These results and those of Bennett and Bartholomew (2) and of Hopkins (5) show, as suggested by Gore (3), that the general rule of Van't Hoff (that the respiration rate doubles or trebles for each 10° C. (18° F.) rise in temperature) does not hold for low temperatures. In general, a greater rate of respiration was found at 36° F. than at 32° or 40° or even at 50° early in the storage season, little relation to sugar concentration being shown at these temperatures. Hopkins (5), working with the Russet Rural variety in storage at various temperatures, reported a more rapid rate of respiration at 32° than at any higher temperature up to 47.6°. In general, the rate of respiration at all temperatures decreased as the storage season advanced, except in the case of the Irish Cobbler variety stored at 60° and 70°, which showed an increase on January 17. Sprouting in this variety, noted soon after this test, may account for the increased respiration. Sprouting was noted in the Green Mountain variety at 60°; but it had apparently just begun, since at this time the growth was not so far advanced as in the Irish Cobbler variety.

COMPOSITION OF INTERNAL ATMOSPHERE

After the three varieties of potatoes had been in storage from November 5 to February 18, the constituents of the atmosphere in the intercellular spaces were determined. Unfortunately, no determinations were made at the beginning of the storage period in order to ascertain what changes took place during storage. However, the results presented in Table 3, representing the average of six tubers, show the composition of the internal atmosphere of each of the three varieties after 105 days of continuous storage at 32°, 36°, 40°, 50°, and 60° F.

TABLE 3.—*Percentage composition of the internal atmosphere of Irish Cobbler, Green Mountain, and Triumph potatoes after storage at various temperatures from November 5 to February 18*

Storage temperature (°F.)	Irish Cobbler			Green Mountain			Triumph		
	CO ₂	O ₂	N	CO ₂	O ₂	N	CO ₂	O ₂	N
32.....	12.0	18.4	69.6	9.0	20.5	70.5	17.2	13.9	68.9
36.....	17.8	15.9	66.3	17.3	16.3	66.4	14.7	18.1	67.2
40.....	12.7	18.0	69.3	10.3	18.4	71.3	9.6	26.3	64.1
50.....	7.2	18.4	74.4	9.9	13.8	76.3
60.....	10.4	17.5	72.1	8.3	17.1	74.6	15.3	18.4	66.3

The internal atmosphere was extracted by the method described and illustrated by Magness (7). For each potato variety, composite samples of gas were obtained from six representative potatoes taken from each storage. A cylindrical plug about 15 mm in diameter was taken from each potato by cutting with a cork borer from side to side through the center. The gas thus obtained was transferred to a Bonnier-Mangin apparatus described by Aubert (1) and Grafe (4, p. 377). The absorption of carbon dioxide was accomplished by using a solution of potassium hydroxide, while oxygen was absorbed with an alkaline solution of pyrogallol. The gas remaining after the carbon dioxide and oxygen had been absorbed was assumed to be nitrogen.

The results obtained are shown in Table 3. Most noticeable is the fact that in the Irish Cobbler and Green Mountain varieties a higher content of CO₂ was found at 36° F. than at any other temperature, whereas in the Triumph variety the CO₂ content was highest at 32°. With a relatively large amount of CO₂ a correspondingly small volume of oxygen was usually found. These results apparently can not be correlated with the relative respiration rate or sugar content at the different temperatures; furthermore, the internal gas composition in all varieties fluctuated with changes in temperature in such a way as to suggest a need for further study in order to determine what relations may exist between the internal atmospheric composition and other physiological reactions.

FURTHER CHANGES IN IRISH COBBLER AND GREEN MOUNTAIN POTATOES AT 60° F., AFTER STORAGE AT 32°

The results shown in Table 1 indicate that potatoes stored at a constant temperature of 32° F. increase in sugar content considerably during storage. The maximum sugar content for all varieties was

found on March 24. On this date Irish Cobbler and Green Mountain potatoes from the lots already described were transferred and held at a constant temperature of 60° for an intensive study of the changes in their sugar content, rate of respiration, and internal atmospheric composition.

SUGAR CONTENT

Samples for sugar determinations were taken on the fourth day after the transfer to storage at 60° F. and frequently thereafter for a period of 20 days, when evidence of sprouting became apparent and further sampling was discontinued. The results presented in Table 4 show a rather uniform decrease in sugars up to the time sampling was discontinued. At this time the total sugar present in the Irish Cobbler variety was still somewhat higher than that found in the fall before the storage experiments started; whereas, in the Green Mountain variety, when sampling was discontinued on April 10, both reducing and total sugars were less than when the experiment started. These data are in agreement with results already well known from earlier investigations on potato storage.

TABLE 4.—*Changes in sugar content of potatoes after being stored at 32° F. from November 5 to March 24 and then transferred to storage at 60°*

Sugars expressed as percentage of dextrose based on fresh weight

Date	Irish Cobbler			Green Mountain		
	Reducing sugar	Sucrose	Total sugar	Reducing sugar	Sucrose	Total sugar
Nov. 5 ^a	0.91	0.27	1.18	1.99	0.27	2.26
Mar. 24.....	4.42	1.45	5.87	4.87	2.41	7.28
Mar. 28.....	3.47	1.00	4.47	3.34	1.26	4.60
Mar. 30.....	3.15	.83	3.98	2.66	.92	3.58
Apr. 2.....	2.50	.68	3.18	2.50	.77	3.27
Apr. 6.....	1.90	.47	2.37	2.30	.67	2.97
Apr. 10.....	1.81	.32	2.13	1.63	.37	2.00
Apr. 17.....	1.61	.30	1.91

^a Before storage.

RESPIRATION

After the potatoes had been transferred to storage at 60° F. and had warmed up to the air temperature (in about 24 hours), samples of approximately 3 kg each were put in the respiration apparatus already described, and the progressive rate of respiration was determined for both varieties by separate runs of varying duration, as shown in Table 5. A previous set of respiration tests was made on Irish Cobbler potatoes after they had been held at 32° only from November 7 to January 17 before being transferred to storage at 60°. It should be noted that sprouting became apparent about nine days after this transfer. These potatoes had been harvested long enough to have passed through their rest period and would naturally sprout at this comparatively high temperature. The results are shown in detail in Table 5.

In both varieties the respiration rate, as indicated by the CO₂ given off per kilogram per hour, showed a tendency to increase during the first few days and then to decrease gradually until it nearly reached that of potatoes held continuously in storage at 60° F. (Table 2.)

This was in general accord with the results reported by Kimbrough (6).

TABLE 5.—*Respiration at 60° F. of two lots of Irish Cobbler and one lot of Green Mountain potatoes, after storage at 32° from November 7 to January 17, November 7 to March 24, and November 7 to March 24, respectively*

Run No.	Respiration test at 60° F. after indicated period of storage at 32°					
	Irish cobbler				Green Mountain	
	Nov. 7 to Jan 17 ^a		Nov. 7 to Mar. 24. ^b		Nov. 7 to Mar. 24 ^b	
	CO ₂ per kilogram per hour	Time in 60°	CO ₂ per kilogram per hour	Time in 60°	CO ₂ per kilogram per hour	Time in 60°
	Mg	Hours	Mg	Hours	Mg	Hours
1.	17.7	49	13.7	66	10.65	66
2.	26.5	92	15.6	139	12.80	139
3.	25.8	139	14.4	235	12.20	235
4.	17.8	217	18.2	310	14.20	310
5.	13.7	289	16.8	478	14.00	478
6.	10.2	406	12.4	526	8.80	526
7.	9.2	601	-----	-----	-----	-----
8.	6.0	765	-----	-----	-----	-----

^a Test was begun Jan. 18 on sample weighing approximately 3 kg.

^b Test was begun Mar. 25 on sample weighing approximately 3 kg.

In the lot of Irish Cobbler potatoes placed at 60° F. on January 17 the increasing rate of respiration for the first few days was followed by a gradual diminution. This increase in respiration in potatoes high in sugar content, removed from a low temperature, is consistent with the results reported by Müller-Thurgau (9). However, the increase in rate lasted longer than he reported (one and one-half to two days), although this may be accounted for by the fact that his investigation was carried on at 68° F. (20° C.) instead of at 60°. The respiration rate in comparable lots of potatoes, that is, potatoes from the same general lots, when harvested and held constantly at 60°, was not determined as late as March 24, because sprouting had already begun by this time. Table 2 shows that on January 17, the respiration rates, as denoted by the CO₂ given off, were 10.40 and 5.80 for the Irish Cobbler and Green Mountain potatoes, respectively. These are less than the respiration rate (17.7) found during the first run in the Irish Cobbler potatoes transferred from storage at 32° to storage at 60° on January 17, and also less than that found in either of the varieties transferred on March 24, when the respiration rates were 13.7 and 10.65, respectively. These values show an increased respiration rate in potatoes moved from storage at 32° to storage at 60°, as reported by previous workers. Comparing the simultaneous respiration activity of potatoes remaining in storage at 32° with that of the lots transferred on March 24 to storage at 60°, the rates were 3.27 and 2.99 (see Table 2) for the Irish Cobbler and Green Mountain varieties, respectively, at 32°, as against 13.7 and 10.65 in the first runs of these varieties after 66 hours at 60°.

COMPOSITION OF INTERNAL ATMOSPHERE

Simultaneously with studies on the changes in sugar content and respiration rate, as reported, changes in internal atmosphere of the tubers were studied. The results are shown in Table 6. Here a marked increase in CO₂ content over the amount present when the potatoes were transferred was found in both varieties on the second day at 60° F. This comparatively high CO₂ content of the internal atmosphere of the tubers persisted for about six days and was correlated with a higher rate of respiration during this period. There was a general, but not always consistent, tendency for the oxygen to decrease as the CO₂ increased.

TABLE 6.—*Change in percentage composition of the internal atmosphere of Irish Cobbler and Green Mountain potatoes at 60° F., after storage at 32° from November 7 to March 24*

Number of days at 60° F.	Irish Cobbler			Green Mountain		
	CO ₂	O ₂	N	CO ₂	O ₂	N
0.....	11.4	19.2	69.4	4.7	47.5	47.8
2.....	32.5	8.5	59.0	23.6	16.1	60.3
4.....	29.7	11.1	59.2	18.1	19.9	62.0
6.....	30.5	26.3	43.2	24.8	18.3	56.9
9.....	20.6	15.6	63.8	16.8	15.2	68.0
13.....	17.5	18.1	64.4	17.7	14.6	67.7
17.....	14.7	16.2	69.1	15.0	16.7	68.3
24.....	12.2	18.5	69.3			

CHANGES IN FROZEN POTATOES

Irish Cobbler and Green Mountain potatoes of the same general lot as already described were taken from continuous storage at 40° F. on March 11 and exposed to a temperature of 22°. They were spread out in a single layer for three hours to bring their internal temperatures to the freezing point or below, and then all were sharply disturbed ("inoculated") by being quickly tossed in a basket and then poured out in a layer as before, each potato being allowed to drop a distance of 3 or 4 inches. This operation was to start simultaneous freezing in order to have approximately the same amount of injury in all specimens when removed. After "inoculation" the tubers were left undisturbed for 2 hours, when they were gathered up and allowed to thaw out at 40° for 24 hours. An examination at this time by cutting various tubers showed mild freezing injury of the net or ring type.

SUGAR CONTENT

After warming at 40° F. for 24 hours, ½-bushel lots of the tubers that had been frozen and similar lots of unfrozen specimens from constant 40° storage were put in storage at 32°, 40°, 50°, 60°, 70°, and 85° for one month. Sugar determinations were then made on samples of about 10 tubers from all lots. (Table 7.) All the analyzed tubers from the lots that had been frozen showed necrosis. Initial sugar determinations were not made, for it was assumed that since the frozen potatoes had remained at the low temperature for only five hours they would show no difference from the unfrozen ones.

TABLE 7.—*Sugar-content changes in frozen and unfrozen potato varieties after one month of storage at various temperatures*

[Sugars expressed as percentage of dextrose based on fresh weight]

IRISH COBBLER						
Storage temperature (°F.)	Frozen ^a			Unfrozen		
	Reducing sugar	Sucrose	Total sugar	Reducing sugar	Sucrose	Total sugar
32	2.78	1.15	3.93	2.52	0.70	3.22
40	1.35	.34	1.69	1.52	.26	1.78
50	1.00	.26	1.26	.94	.24	1.18
60	.87	.11	.98	.79	.26	1.05
70	.72	.86	1.58	.53	.82	1.35
85	.53	.34	.87	.39	.09	.48

GREEN MOUNTAIN						
Storage temperature (°F.)	Frozen ^a			Unfrozen		
	Reducing sugar	Sucrose	Total sugar	Reducing sugar	Sucrose	Total sugar
32	2.98	1.80	4.78	2.98	1.13	4.11
40	1.65	.41	2.06	1.69	.48	2.17
50	1.13	.20	1.33	1.20	.23	1.43
60	1.15	.20	1.35	.80	.20	1.09
70	.75	.40	1.15	.57	.41	.98
85	.83	.84	1.67	.44	.11	.55

^a The frozen potatoes were held at a temperature of 22° F. for 3 hours, inoculated by being jarred, and then allowed to freeze for 2 hours, after which they were held at 40° for 24 hours to warm up.

These results did not consistently prove or disprove the common belief that frozen potatoes are sweeter than unfrozen ones. At some holding temperatures the sugar content of the frozen potatoes was somewhat higher than that of unfrozen potatoes, and at other temperatures the reverse was true; moreover, the same relations did not hold for both varieties. Much of this lack of correlation can be explained by the possibility that different degrees of freezing occurred and caused variation among individual specimens. The inference that in most cases there was a slightly higher sugar content in potatoes that had been frozen is consistent with the slightly higher rate of respiration shown later.

RESPIRATION

Approximately 3-kg duplicate lots of frozen Irish Cobbler potatoes from the lot described were transferred to storage at 60° F., after they had thawed out at 40° for 24 hours, and were left for about 12 hours to reach this temperature, along with similar lots from the constant storage at 40°. These lots of potatoes were then put in the respiration apparatus and the comparative rates of respiration of frozen and unfrozen potatoes determined in three runs, as shown in Table 8. In every run a somewhat higher respiration rate was found in the frozen potatoes than in the unfrozen ones from the same general source.

TABLE 8.—*Respiration at 60° F. of frozen and unfrozen Irish Cobbler potatoes*

Run No.	Frozen *		Unfrozen	
	CO ₂ per kilogram per hour	Duration of run	CO ₂ per kilogram per hour	Duration of run
	<i>Mg</i>	<i>Hours</i>	<i>Mg</i>	<i>Hours</i>
1.....	16.6	72	9.7	72
2.....	15.2	72	12.1	72
3.....	11.1	120	9.3	120

* The frozen potatoes were held at 22° for 3 hours, inoculated by being jarred, and then allowed to freeze for 2 hours, after which these, as well as the unfrozen potatoes, were placed at 60° to warm up overnight

SUMMARY

Large representative lots of Irish Cobbler, Green Mountain, and Triumph potatoes were stored continuously at 32°, 36°, 40°, 50°, 60°, and 70° F. from November 5 until March 24, and periodic determinations were made of sugar content and respiration rate at these temperatures. Analyses of the internal or intercellular atmosphere were also made after holding the potatoes in storage at these temperatures from November 5 until February 18.

At 32° F. the sugar content steadily increased in all varieties until March 24. After this, further analyses were not made until June 1, when a slight decrease was shown. At 36° there was a marked increase in sugar, but to a less extent than at 32°. At 40° the periodic results were fluctuating, but on the whole there was a slight increase in sugar. At 50°, 60°, and 70° the sugar content decreased steadily in proportion as the temperature increased.

Respiration of Irish Cobbler and Green Mountain potatoes increased at successively higher temperatures above 40° F. Respiration at 36° was more rapid than at 40° or 32°. In general the respiration rate decreased as the storage period increased.

The CO₂ content of the internal atmosphere of Irish Cobbler and Green Mountain tubers was greatest at 36° F., and that of the Triumph variety was greatest at 32°. At the other temperatures no apparent correlation existed between the internal atmospheric composition and the temperature.

After Irish Cobbler and Green Mountain potatoes had been in storage at 32° F. until March 24, some of each of the varieties were removed and held at 60°; sugar content, respiration rate, and composition of the internal atmosphere were then determined at frequent intervals.

The sugar content steadily and rapidly decreased, while the rate of respiration at first increased and then decreased. The CO₂ content of the internal atmosphere increased rapidly during the first two days and remained high for about six to nine days before decreasing.

Irish Cobbler and Green Mountain potatoes, after being in storage at 40° F. until March 11, were slightly frozen at 22° for five hours. They were then thawed at 40°, divided, and stored at 32°, 40°, 50°, 60°, 70°, and 85°, along with unfrozen lots from the same temperature. After a month's storage sugar determinations were made. At most temperatures the potatoes that had been frozen showed a somewhat higher sugar content than did the unfrozen potatoes.

Samples of the frozen potatoes, after thawing for 24 hours at 40° F., together with unfrozen potatoes from storage at 40°, were stored at 60° for 24 hours so as to allow their temperature to come to room temperature. The rate of respiration was then determined in both lots. A somewhat higher rate was found in the frozen potatoes.

LITERATURE CITED

- (1) AUBERT, E.
1891. NOUVEL APPAREIL DE MM. G. BONNIER ET L. MANGIN POUR L'ANALYSE DES GAZ. *Rev. Gén. Bot.* 3:97-104, illus.
- (2) BENNETT, J. P., and BARTHOLOMEW, E. T.
1924. THE RESPIRATION OF POTATO TUBERS IN RELATION TO THE OCCURRENCE OF BLACKHEART. *Calif. Agr. Expt. Sta. Tech. Paper* 14, 35 p., illus.
- (3) GORE, H. C.
1911. STUDIES ON FRUIT RESPIRATION. I. THE EFFECT OF TEMPERATURE ON THE RESPIRATION OF FRUITS. II. THE EFFECT OF PICKING ON THE RATE OF EVOLUTION OF CARBON DIOXID BY PEACHES. III. THE RATE OF ACCUMULATION OF HEAT IN THE RESPIRATION OF FRUIT UNDER ADIABATIC CONDITIONS. *U. S. Dept. Agr., Bur. Chem. Bul.* 142, 40 p., illus.
- (4) GRAFE, V.
1914. ERNÄHRUNGSPHYSIOLOGISCHES PRAKTIKUM DER HÖHEREN PFLANZEN. 494 p. Berlin.
- (5) HOPKINS, E. F.
1924. RELATION OF LOW TEMPERATURES TO RESPIRATION AND CARBOHYDRATE CHANGES IN POTATO TUBERS. *Bot. Gaz.* 78:[311]-325, illus.
- (6) KIMBROUGH, W. D.
1925. A STUDY OF RESPIRATION IN POTATOES WITH SPECIAL REFERENCE TO STORAGE AND TRANSPORTATION. *Md. Agr. Expt. Sta. Bul.* 276, p. [51]-72, illus.
- (7) MAGNESS, J. R.
1920. COMPOSITION OF GASES IN INTERCELLULAR SPACES OF APPLES AND POTATOES. *Bot. Gaz.* 70:308-316, illus.
- (8) ——— and DIEHL, H. C.
1924. PHYSIOLOGICAL STUDIES OF APPLES IN STORAGE. *Jour. Agr. Research* 27:1-38, illus.
- (9) MÜLLER-THURGAU, H.
1880-1886. UEBER DAS GEFRIEREN UND ERFRIEREN DER PFLANZEN. *Landw. Jahrb.* 9:[133]-189, 1880; 15:[453]-610, illus. 1886.

APPARENT DIGESTIBILITY OF, AND NITROGEN, CALCIUM, AND PHOSPHORUS BALANCE OF DAIRY HEIFERS ON, ARTIFICIALLY DRIED PASTURE HERBAGE¹

By R. E. HODGSON, *Agent, Bureau of Dairy Industry, United States Department of Agriculture, Dairy Husbandman, Western Washington Experiment Station, and Assistant in Dairy Husbandry, Washington Agricultural Experiment Station,* and J. C. KNOTT, *Superintendent of Official Testing, Washington Agricultural Experiment Station*²

INTRODUCTION AND REVIEW OF PREVIOUS INVESTIGATIONS

The value of an intensive system of pasture management in increasing the yield and nutritive value of pasture herbage has been amply demonstrated by various investigators.

Results obtained by Grunder³ in a study of the yield and chemical composition of mixed pasture grasses and clovers show that under a system of biweekly cuttings the dry matter of the herbage possessed an average percentage composition of 26.28 crude protein, 17.52 crude fiber, 40.72 nitrogen-free extract, 3.31 ether extract, 12.17 total ash, 0.57 phosphorus, and 0.66 calcium.

Ellenberger (1)⁴ and others reported that the dry matter of ordinary pasture grass in Vermont had an average composition of 19.05 per cent crude protein, 17.87 per cent crude fiber, 48.31 per cent nitrogen-free extract, 0.854 per cent calcium, and 0.270 per cent phosphorus. They concluded that quantity rather than quality was the dominating factor affecting carrying capacity of closely grazed pastures.

Woodman and his associates (7, 8, 9, 10, 11, 12, 13) reported, from an extensive study of the nutritive value of pastures, that young pasture grass obtained under a weekly, biweekly, triweekly, or monthly system of cutting was, in terms of its digestible nutrient, a "watered concentrate" rather than a roughage. Seventy-two per cent of the organic matter as well as 80 per cent of the small amount of fiber present in the grass was digestible. The immature herbage contained approximately 20 per cent digestible crude protein.

The proper preservation of large amounts of this highly nutritious feed without affecting its feeding value is a matter of much interest at the present time. With the advent of the commercial hay drier, it seems possible that immature pasture herbage possessing a highly digestible protein content might be harvested, artificially dried, and fed to dairy cattle as a protein-rich concentrate. Reports of the digestibility of artificially dried grasses would seem to indicate that the process of drying does not greatly depress their feeding value.

Woodman and others (11) have shown that young grass does not suffer any appreciable depression in digestibility when artificially

¹ Received for publication February 16, 1932, issued November 1932. Scientific Paper No. 218, College of Agriculture and Experiment Station, State College of Washington.

² The writers are indebted to Harold Gerritz, who was responsible for all the analytical work involved, and to M. S. Grunder, under whose direction the herbage was collected and preserved.

³ Unpublished data obtained from M. S. Grunder, agronomist, Western Washington Experiment Station Puyallup, Wash.

⁴ Reference is made by number (italic) to Literature Cited. p. 562.

dried at the temperature of steam or by direct heat in a kiln. The digestibility coefficients of the total organic matter and of the crude protein portion of the kiln-dried grass were 79.44 and 78.24, respectively.

Watson (6) has presented data showing that sheep on an exclusive diet of artificially dried pasture grass were able to maintain their live weight. The digestibility coefficients of the organic matter and crude protein of the dried grass were 76.36 and 76.35, respectively. The sheep maintained positive nitrogen, calcium, phosphorus, and potassium balances throughout the trial.

Watson (6) fed artificially dried pasture grass to milking dairy cows and found it to be very palatable. It proved to produce the quantity of milk for which the nutrients it supplied were theoretically capable.

During the winter of 1931 the apparent digestibility of artificially dried pasture herbage was studied at this station. Dairy heifers were used as the experimental animals. In conjunction with this work, it was thought advisable to determine the balance of nitrogen, calcium, and phosphorus of heifers on an exclusive diet of artificially dried pasture herbage.

EXPERIMENTAL PROCEDURE

The artificially dried pasture herbage was obtained from plots of pure stands of the following grasses and clovers: English ryegrass (*Lolium perenne*), Italian ryegrass (*L. multiflorum*), orchard grass (*Dactylis glomerata*), rough-stalked meadow grass (*Poa trivialis*), Kentucky bluegrass (*P. pratensis*), annual bluegrass (*P. annua*), meadow fescue (*Festuca elatior*), creeping bent (*Agrostis stolonifera*), ladino clover (*Trifolium repens latum*), alsike clover (*T. hybridum*), and white clover (*T. repens*). These grasses and clovers were cut with a lawn mower at biweekly intervals and dried in an experimental drier and mixed. The material was cut into approximately 3-inch lengths. The predominating plants in the resulting mixture were English ryegrass and Italian ryegrass.

Drying was done in a small batch drier. Heated air passing through the oven at from 100° to 200° F. dried the material to approximately 5 per cent moisture in about 12 hours. The dried herbage retained its green color and possessed a pleasing aroma.

The usual precautions necessary in conducting a digestibility and mineral-balance study as outlined by Forbes and Grindley (3, p. 17-27) were observed throughout the experiment. The experimental animals were three Holstein heifers, approximately 2 years old and weighing on an average about 950 pounds each. They were gradually changed to the experimental ration prior to going into the digestion stalls at the beginning of the preliminary period.

An amount of artificially dried pasture herbage sufficient to last throughout the experiment was spread out on a tight floor, thoroughly mixed, and divided into three piles. The individual feeds for the entire period were weighed out into paper bags; a part of each feed being taken from each of the three piles. After five portions had been weighed, a small amount of the feed was taken from each of the three piles to form a composite sample for chemical analysis.

The daily ration used was calculated to satisfy the total digestible nutrient requirements of growing dairy animals as suggested by Fitch

and Lush (2). Watson's (6) digestibility figures for artificially dried grass were used as a basis for calculating these rations. The feed proved to be very palatable, and throughout the experiment all that was offered the animals was readily eaten. Distilled water was available to the animals at all times. Three-fourths of an ounce of chemically pure sodium chloride was fed daily.

The preliminary feeding period lasted 10 days, the collection period 15 days. The experimental day began at 3.00 p. m., at which time the live weights of the heifers were recorded. Feeding was done at 4.30 p. m. and 5.30 a. m. The animals were exercised at 10 a. m. daily.

The urine and feces were collected by three attendants who worked in 8-hour shifts. The urine was caught in suitable containers and placed in bottles. The 24-hour sample from each heifer was weighed, thoroughly mixed, and an aliquot of 10 per cent was taken and analyzed daily for nitrogen, calcium, and phosphorus.

The feces were caught in pails and transferred to covered, galvanized-iron cans. At the close of each experimental day the feces were weighed and mixed, and a representative 5 per cent sample was placed in a friction-top lard pail, sealed, and stored in a refrigerator at -10° F. At the close of the collection period, the aliquot samples representing the total feces collected from each animal were mixed, ground, remixed, and sampled for chemical analysis.

The analyses of the feed, urine, and feces were carried out by the usual standard methods. Determinations of iron and silica were made on the feed and feces for the purpose of comparing the apparent digestibility of the various constituents of the feed as determined by the usual method with the modified procedure suggested by Gallup and Kuhlman (4).

EXPERIMENTAL DATA

The results of this investigation are presented in four tables. The amount of artificially dried herbage consumed and the feces and urine voided during the experimental period are given in Table 1. Table 2 shows the percentage composition of the feed, feces, and urine. In Table 3 are shown the quantities of the various feed constituents ingested, voided, and retained by each heifer during the 15-day collection period. This table also shows the apparent digestibility in percentage of the feed constituents as determined under the conditions of this experiment.

TABLE 1.—*Feed consumed and feces and urine voided (grams) during 15-day metabolism trial*

Heifer No.	Feed consumed	Feces voided	Urine voided
107-----	102, 270	125, 204	227, 623
108-----	102, 270	156, 094	203, 795
109-----	102, 270	133, 090	247, 918

TABLE 2.—Percentage composition of feed, feces, and urine

(Dry-matter basis)

Heifer No. and material analyzed	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber	Ash	Nitrogen	Calcium	Phosphorus
107:									
Feces.....	21.71	18.81	30.72	8.14	15.73	23.60	3.01	2.39	1.87
Urine.....							1.174	.002	.012
108:									
Feces.....	19.66	18.31	30.87	8.24	15.16	23.43	2.93	3.29	1.87
Urine.....							1.270	.004	.005
109:									
Feces.....	20.49	20.12	30.66	8.81	14.86	22.80	3.22	2.32	1.94
Urine.....							1.032	.002	.009
All 3 heifers: Feed.....	20.05	24.64	39.19	8.50	18.09	11.18	3.04	.78	.65

TABLE 3.—Apparent digestibility of artificially dried pasture herbage fed to each animal

HEIFER 107

Item	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber	Ash
Ingested..... grams..	88,003.34	21,684.02	34,488.51	3,080.12	15,919.80	9,838.77
Voided..... do..	27,181.79	5,112.89	8,350.25	2,212.60	4,275.70	6,414.90
Digested..... do..	60,821.55	16,571.13	26,138.26	867.52	11,644.10	3,423.87
Digested..... per cent..	69.11	76.42	75.79	28.16	73.14	34.80

HEIFER 108

Item	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber	Ash
Ingested..... grams..	88,003.34	21,684.02	34,488.51	3,080.12	15,919.80	9,838.77
Voided..... do..	30,489.51	5,582.63	9,412.11	2,542.82	4,622.21	7,143.69
Digested..... do..	57,513.83	16,101.39	25,076.40	537.30	11,297.59	2,695.08
Digested..... per cent..	65.35	74.25	72.71	17.44	70.97	27.39

HEIFER 109

Item	Dry matter	Crude protein	Nitrogen-free extract	Ether extract	Crude fiber	Ash
Ingested..... grams..	88,003.34	21,684.02	34,488.51	3,080.12	15,919.80	9,838.77
Voided..... do..	27,935.59	5,620.64	8,565.05	2,461.12	4,151.23	6,369.31
Digested..... do..	60,067.75	16,063.38	25,923.46	618.00	11,768.57	3,469.46
Digested..... per cent..	68.26	74.08	75.16	20.10	73.92	35.26
Average percentage digested by all 3 heifers.....	67.57	74.92	74.55	21.90	72.68	32.48

The digestibility coefficients in all cases, with the exception of the ether extract and ash, are in close agreement for the three heifers. The average digestibility coefficient of 74.92 for crude protein gave this artificially dried pasture herbage a digestible crude-protein content of more than 18 per cent, which compares favorably with some of the higher protein concentrates.

The dried herbage had a digestible carbohydrate equivalent of 45.63 per cent and a total digestible nutrient content of 64.74 per cent. The high digestibility of the crude fiber contributes materially to the feeding value of this feed.

The average digestibility coefficient of 21.9 for the ether extract as obtained in this experiment is considerably lower than the 59.6 per cent obtained by Watson (5) or the 70.6 per cent obtained by Woodman and his associates (7). Not only was the digestibility

of the ether extract low in this experiment, but considerable variation was shown by individual animals. It is possible that the method of drying the feed may have depressed the digestibility of the ether extract.

Heifer 108 had the lowest coefficient of digestibility for each constituent except protein. This heifer was in much better condition than either heifer 107 or 109. The digestibility of artificially dried pasture herbage as determined in this experiment with dairy heifers was uniformly lower than that obtained by Watson (6) or by Woodman et al. (11) with sheep.

The average daily balance of nitrogen, calcium, and phosphorus for each of the three heifers is given in Table 4. A slight negative

TABLE 4.—Average daily nitrogen, calcium, and phosphorus balances (in grams) for the three heifers during 15-day metabolism trial

Heifer No.	Nitrogen			Calcium			Phosphorus		
	Fed	Eliminated	Balance	Fed	Eliminated	Balance	Fed	Eliminated	Balance
107.....	231.16	232.68	-1.52	45.57	43.67	+1.90	38.25	35.64	+2.61
108.....	231.16	232.12	-.96	45.57	67.45	-21.88	38.25	38.74	-.49
109.....	231.16	230.58	+5.58	45.57	43.53	+2.04	38.25	35.79	+2.47
Average balance.....			-63			-5.98			+1.53

average daily nitrogen balance may indicate that some of the protein ingested was used for the production of energy. According to the apparent digestibility figures obtained in this experiment, the ration fed these animals furnished an excess of protein but did not furnish sufficient energy. In spite of the insufficient supply of energy in the ration, each of the three heifers made substantial gains in live weight throughout the collection period, which would further indicate the utilization of protein material for the production of energy.

Heifer 107 had an average initial live weight of 940 pounds and an average final weight of 952.8 pounds, indicating a gain of 12.8 pounds for the 15-day collection period. Heifer 108 had an average initial live weight of 1,023.6 pounds and an average final weight of 1,048.6 pounds, indicating a gain of 25 pounds during the collection period. Heifer 109 weighed 875.8 pounds initially, 897.2 pounds finally, and gained 21.4 pounds. The average daily gain in live weight per animal was 1.32 pounds.

Heifer 109 showed a slight positive daily nitrogen balance. This was the smallest and poorest-conditioned animal of the three. It is probable that the energy content of the ration more nearly supplied the energy requirements of this heifer and, therefore, not so much of the protein digested was used for the production of energy.

Heifers 107 and 109 showed a positive average daily calcium balance of 1.90 g and 2.04 g, respectively, while heifer 108 had a negative average daily calcium balance of 21.88 g. Likewise heifers 107 and 109 had a positive daily phosphorus balance of 2.61 g and 1.53 g, while heifer 108 had a negative daily balance of 0.49 g. The average daily intake of calcium per animal was 45.57 g and of phosphorus 38.25 g, which gives a calcium-phosphorus ratio of 1.2:1.

Lindsey and his associates (5) were able to secure normal growth of Holstein heifers in their third year with an average daily intake of 2.5 g of calcium per 100 pounds live weight. They found that the calcium and phosphorus was retained at the approximate ratio of 2 to 1. On the basis of the above findings, the heifers in this experiment were furnished with an abundance of both calcium and phosphorus. However, the retention ratio with the two animals having positive calcium and phosphorus balances was about 0.78 calcium to 1 of phosphorus. With a low calcium and phosphorus retention in the case of two animals and a negative balance in the case of the third, together with an unusual calcium and phosphorus retention ratio, there are apparently some factors that have interfered with normal retention of these constituents in this experiment.

SUMMARY

Metabolism experiments with three 2-year-old Holstein heifers were conducted to determine the apparent digestibility of artificially dried pasture herbage and to determine the nitrogen, calcium, and phosphorus balances of these heifers on an exclusive diet of dried herbage.

The crude protein, which constituted 24.64 per cent of the dry matter of the artificially dried pasture herbage, had an average digestibility coefficient of 74.92 per cent.

The average digestibility of the nitrogen-free extract was 74.55 per cent.

The digestibility of the ether extract was rather low, the average for the three heifers being only 21.90 per cent. This low digestibility of the ether extract may possibly have been caused by the drying process.

The crude fiber was found to be 72.68 per cent digestible. The digestible crude fiber contributes much to the feeding value of artificially dried pasture herbage.

The nitrogen balances were negative in two of the three cases with an average negative balance of 0.63 g daily.

Two heifers showed a positive daily calcium balance of 1.90 g and 2.04 g, respectively, while the third had a negative daily balance of 21.88 g. This latter animal also showed a negative phosphorus balance of 0.49 g daily, while the other two had positive balances of 2.61 g and 1.53 g, respectively.

The average daily intake of calcium was 45.57 g and of phosphorus 38.25 g, which gives a calcium-phosphorus ratio of 1.2:1.

LITERATURE CITED

- (1) ELLENBERGER, H. B., NEWLANDER, J. A., and JONES, C. H.
1929. YIELD AND COMPOSITION OF PASTURE GRASS. *Vt. Agr. Expt. Sta. Bul.* 295, 68 p., illus.
- (2) FITCH, J. B., and LUSH, R. L.
1931. AN INTERPRETATION OF THE FEEDING STANDARDS FOR GROWING DAIRY CATTLE. *Jour. Dairy Sci.* 14: 116-124, illus.
- (3) FORBES, E. B., and GRINDLEY, H. S.
1923. ON THE FORMULATION OF METHODS OF EXPERIMENTATION IN ANIMAL PRODUCTION. *Bul. Natl. Research Council* 6 (pt. 2, no. 33), 54 p.
- (4) GALLUP, W. D., and KUHLMAN, A. H.
1931. A PRELIMINARY STUDY OF THE DETERMINATION OF THE APPARENT DIGESTIBILITY OF PROTEIN BY MODIFIED PROCEDURES. *Jour. Agr. Research* 42:665-669.

- (5) LINDSEY, J. B., ARCHIBALD, J. G., and NELSON, P. R.
1931. THE CALCIUM REQUIREMENTS OF DAIRY HEIFERS. *Jour. Agr. Research* 42:883-896, illus.
- (6) WATSON, S. J.
1931. INVESTIGATIONS INTO THE INTENSIVE SYSTEM OF GRASSLAND MANAGEMENT. IV. THE DIGESTIBILITY AND FEEDING VALUE OF ARTIFICIALLY DRIED GRASS. *Jour. Agr. Sci. [England]* 21: 414-424, illus.
- (7) WOODMAN, H. E., BLUNT, D. L., and STEWART, J.
1926. NUTRITIVE VALUE OF PASTURE. I. SEASONAL VARIATIONS IN THE PRODUCTIVITY, BOTANICAL AND CHEMICAL COMPOSITION, AND NUTRITIVE VALUE OF MEDIUM PASTURAGE ON A LIGHT SANDY SOIL. *Jour. Agr. Sci. [England]* 16:[205]-274, illus.
- (8) ——— BLUNT, D. L., and STEWART, J.
1927. NUTRITIVE VALUE OF PASTURE. II. SEASONAL VARIATIONS IN THE PRODUCTIVITY, BOTANICAL AND CHEMICAL COMPOSITION, AND NUTRITIVE VALUE OF PASTURAGE ON A HEAVY CLAY SOIL. *Jour. Agr. Sci. [England]* 17:[209]-263, illus.
- (9) ——— NORMAN, D. B., and BEE, J. W.
1928. NUTRITIVE VALUE OF PASTURE. - III. INFLUENCE OF THE INTENSITY OF GRAZING ON THE YIELD, COMPOSITION, AND NUTRITIVE VALUE OF PASTURE HERBAGE (PART I). *Jour. Agr. Sci. [England]* 18: [266]-294.
- (10) ——— NORMAN, D. B., and BEE, J. W.
1929. NUTRITIVE VALUE OF PASTURE. IV. THE INFLUENCE OF THE INTENSITY OF GRAZING ON THE YIELD, COMPOSITION, AND NUTRITIVE VALUE OF PASTURE HERBAGE (PART II). *Jour. Agr. Sci. [England]* 19:[236]-265.
- (11) ——— BEE, J. W., and GRIFFITH, G.
1930. NUTRITIVE VALUE OF PASTURE. V. PASTURE GRASS CONSERVATION: THE INFLUENCE OF ARTIFICIAL DRYING ON THE DIGESTIBILITY OF PASTURE HERBAGE. *Jour. Agr. Sci. [England]* 20:[53]-62.
- (12) ——— and EVANS, R. E.
1930. NUTRITIVE VALUE OF PASTURE. VI. THE UTILIZATION BY SHEEP OF MINERAL-DEFICIENT HERBAGE. *Jour. Agr. Sci. [England]* 20: [586]-[617].
- (13) ——— NORMAN, D. B., and FRENCH, M. H.
1931. NUTRITIVE VALUE OF PASTURE. VII. THE INFLUENCE OF INTENSITY OF GRAZING ON THE YIELD, COMPOSITION, AND NUTRITIVE VALUE OF PASTURE HERBAGE (PART III). *Jour. Agr. Sci. [England]* 21:[267]-[323].

AN EMPIRICAL TEST OF THE APPROXIMATE METHOD OF CALCULATING COEFFICIENTS OF INBREEDING AND RELATIONSHIP FROM LIVESTOCK PEDIGREES¹

By JAY L. LUSH

Head, Animal Breeding Subsection, Animal Husbandry Section, Iowa Agricultural Experiment Station

INTRODUCTION

In 1925² Wright and McPhee reported a sampling method for calculating inbreeding coefficients and relationship coefficients from random ancestral lines of livestock pedigrees. In applying this method to a study of the genetic history of the Rambouillet breed of sheep the writer had occasion to make some empirical tests of its reliability.

PLAN OF STUDY

The study involved a series of samples, each consisting of 400 pedigrees of sheep registered as having been born in a certain year. The pedigrees in each sample were systematically selected at regular intervals in the appropriate volume of the flock book, without regard to breeder, geography, or individual merit. From each of these 400 pedigrees two random lines of ancestry, one from the sire and one from the dam, were traced back to the beginning of the flock book, each sequence of sires or dams being determined at random. The samples from two years (1916 and 1926) were duplicated to make the findings of the study more reliable and to test certain apparent discrepancies in the genetic trends from period to period. A study of the differences between the two 1916 samples and the differences between the two 1926 samples is the basis of the present study. In taking the duplicate samples, animals already used in the first sample were avoided and, as far as possible, animals for the second sample were taken from pages other than those from which the first sample animals had been taken.

The second 1916 sample did not agree at all with the first in inbreeding coefficients, although it did agree fairly well in relationship coefficients. The difference in inbreeding coefficients was a little more than four times its probable error. This led to a test to determine whether the difference lay in the method of sampling the breed or in the method of sampling the ancestral lines of the pedigrees after the pedigrees had been selected. Using the very same animals selected in the first 1916 sample, the writer traced back new random lines from the sires and dams. The results were in almost identical agreement with the findings of the second sample rather than with the random lines first run from the first sample. To complete this test, new random lines were traced back from the sires and dams of

¹ Received for publication Feb. 22, 1932; issued November, 1932. Journal Paper No. B38 of the Iowa Agricultural Experiment Station.

² WRIGHT, S. and MCPHEE, H. C. AN APPROXIMATE METHOD OF CALCULATING COEFFICIENTS OF INBREEDING AND RELATIONSHIP FROM LIVESTOCK PEDIGREES. *Jour. Agr. Research* 31:377-383. 1925.

the animals in the second sample of 400 pedigrees. Again there resulted almost identical agreement with the first sampling of those pedigrees and with the second sampling of the first 1916 pedigrees. In the second trials of the two 1916 samples only the inbreeding coefficients were studied.

INBREEDING COEFFICIENTS

The results of the tests are shown in Table 1. One of the four 1916 trials was so far out of line with the other three that it demanded an explanation. Only seven different ancestors furnished ties in both of the 1926 samples. Four different ancestors each furnished one or more ties for each of the four 1916 trials, 6 ancestors furnished ties in both trials of the second 1916 sample, and 17 of the 28 different ancestors that furnished ties in the second trial of the first 1916 sample had also furnished ties in the first trial of that sample.

TABLE 1.—Agreement between duplicate samples, each of four hundred 2-column pedigrees of Rambouillet sheep

Item	Sheep born in 1926		Sheep born in 1916			
	First sample	Second sample	First sample		Second sample	
			First trial	Second trial	First trial	Second trial
Inbreeding:						
Ties found.....	41	44	53	29	29	29
Number of different ancestors on which the ties occurred.....	38	41	41	28	28	27
Coefficient.....	5.3±0.5	5.6±0.5	6.8±0.6	3.7±0.4	3.7±0.4	3.7±0.4
Inter se relationship:						
Ties found.....	11	10	9		12	
Coefficient.....	2.7±0.5	2.5±0.5	2.1±0.5		3.0±0.6	
Relationship to individual animals:						
Registry No.—						
328.....	2.3	1.4	2.5		3.5	
922.....	4.1	5.4	4.8		5.9	
923.....	2.8	3.6	1.9		2.3	
952.....	4.4	4.6	5.0		4.8	
961.....	5.9	8.2	5.3		6.9	
1230.....	1.7	2.3	2.4		1.4	
1245.....	.9	1.3	1.2		1.1	
1261.....	2.0	3.0	3.4		1.5	
1268.....	2.1	3.2	3.2		2.0	
2937.....	3.6	4.3	5.4		5.4	
2938.....	.5	.6	.5		.4	
2940.....	2.3	2.6	2.4		2.6	
13402.....	5.7	6.9	5.4		6.2	
13405.....	3.8	1.6	3.0		2.5	
13406.....	3.4	2.4	3.4		3.9	
13411.....	4.7	3.7	4.6		5.2	
13420.....	4.4	5.7	6.8		5.4	
13435.....	5.4	6.6	7.9		6.4	
13436.....	3.7	5.0	8.5		6.1	
13442.....	6.6	6.2	7.9		8.1	
13454.....	5.6	6.2	6.0		6.1	
17519.....	6.3	4.6	6.2		6.2	
20079.....	3.6	2.4	2.7		1.1	
29311.....	4.9	6.4				
29516.....	6.1	5.8	6.5		6.8	
31522.....	3.8	2.4				
34671.....	5.0	5.6				
37853.....	5.9	4.9				
39516.....	2.8	2.8	1.2		.6	
39836.....	6.9	8.6	6.2		5.5	
52810.....	5.5	5.1	5.5		6.0	
69456.....	5.0	6.9				
98869.....	6.7	7.2				

p. The random sequences were obtained in these studies by tossing a penny for each sequence instead of establishing a chain of some thousand sequences and following that chain through again and again, as was suggested by Wright and McPhee. It seems certain that the discrepancy in the first trial of the first 1916 sample arose on account of a systematic error that inadvertently crept in through the use of this method. When a new sequence was desired, a penny was drawn from the box and laid on the table and the sire or dam was selected according to whether head or tail of the penny was up. When the first trial of the first 1916 sample was run, the two lines from a single pedigree were carried back simultaneously, as it was often possible to look up two pedigrees in a single volume of the flock book.

Evidently the person doing this work often laid a penny on the table, determined a sequence from it, and then in looking up the next sequence in the other line forgot that the penny lying there had already been used in determining the previous sequence. Thus he often used a single penny throw for two sequences from the same volume of the flock book and in the two lines running back from a single pedigree. Part of the evidence for this is the fact that in this trial an unusual number of ties were found on very recent ancestors in pedigrees taken from consecutive or almost consecutive pages of the flock book, and on ancestors which did not often appear at all in other pedigrees. In the Rambouillet flock book there are often several consecutive pages of pedigrees registered from a single flock. If in such a flock the breeder was rather extensively using the son of one ram on daughters or granddaughters of the same ram, and if the person tracing the two lines simultaneously from the same pedigree often forgot and used the same penny throw a second time, the results actually found would have been almost inevitable. Additional evidence in favor of this explanation comes from the fact that others working with this method in this laboratory often found themselves consciously in doubt as to whether they had just used or were just getting ready to use a given penny in the tracing of these lines. When the worker was aware of this, the doubtful penny was discarded, but a worker not constantly on guard might often use the same penny throw twice. The systematic error that would result would not affect the relationship coefficients materially. Of course there is no reason to expect often such remarkably close agreement as was actually observed between the other three 1916 trials.

RELATIONSHIP COEFFICIENTS

On the advice of Doctor Wright a slight departure was made from the method outlined by Wright and McPhee.³ In calculating the inter se relationship within the sample of 400 pedigrees, one line from one pedigree was matched with another line chosen at random from among all the other pedigrees. When a line was once used in this matching process, it was marked and was not again used for this purpose. Thus from four hundred 2-column pedigrees there were 400 matchings of one line against one line, no line being used a second time.

³ Wright has called attention to an error in the printed statement of Wright and McPhee's method in the twelfth line on p. 383 which reads: "A tie in a comparison of four-column pedigrees (four possible ties) * * *." The word "four-column" should be "two-column."

The relationship of individual animals to the entire breed is made up partly of direct relationship and partly of collateral relationship. For example, a given animal, B, may be found as an ancestor in 16 out of a possible 800 lines in a sample. That is direct relationship. In addition to this, B's sire and dam may perhaps be found in 12 more lines which do not trace to them through B but through some of B's half or full brothers or sisters. This would bring about a certain amount of genetic resemblance between B and the entire breed by collateral relationship. More remote ancestors may also contribute to this relationship, although of course the additional contribution made by another collateral appearance of an ancestor is approximately halved with each generation farther back the ancestor is in the pedigree.

The probable errors for the coefficient of relationship between an individual and the entire breed will depend in part on how much of the relationship is direct and how much is collateral. The collateral relationship is spread out over a larger number of ancestors, and the probable error contributed by finding or failing to find a particular ancestor decreases as that ancestor becomes more remote. The maximum probable errors for these relationship coefficients occur where all the relationship is direct. The following probable errors are approximately correct if one-half of the total relationship found is direct, one-fourth of the total amount is collateral through the two parents, and the remaining one-fourth is collateral through the four grandparents. On the whole, this gives probable errors that are smaller than they should be for most of the individual animals shown in Table 1. Consequently the differences between the coefficients from duplicate samples in Table 1 will, when compared with these probable errors, appear more significant than they really are. The approximate probable error of the difference between two coefficients of relationship, each calculated from a sample of 800 random lines, is as follows:

Size of coefficient	Probable error of difference between two such coefficients	Size of coefficient	Probable error of difference between two such coefficients
<i>Per cent</i>		<i>Per cent</i>	
2	0.40	6	0.68
3	.48	7	.73
4	.55	8	.78
5	.62	9	.82

When these approximate probable errors are compared with the 60 differences between the coefficients for the same animal in duplicate samples, 22, or 37 per cent, of those differences are less than these probable errors; 35 per cent of them exceed twice these probable errors, and 5, or 8 per cent, of them exceed three times these probable errors.

The probable errors were calculated exactly for these five largest differences, and it was found that two were really less than three times their probable errors, while the other three (20079 in 1916, 1261 in 1916, and 13405 in 1926) were 3.4, 3.7, and 4.0 times their respective probable errors. In a group of 60 differences one would expect two or three to exceed the limits of three times their probable errors just as a matter of chance.

Figure 1 shows graphically the agreement between the number of times ancestors were found in the first and second 1926 samples. All ancestors found as many as five times in either sample are included. Those not found five times in at least one sample are certainly not important. In general, close agreement is evident. Since the number of times an ancestor is found is not entirely independent of the number of times each of its descendants or ancestors is found, it was not thought worth while to reduce the facts shown in Figure 1 to mathematical terms.

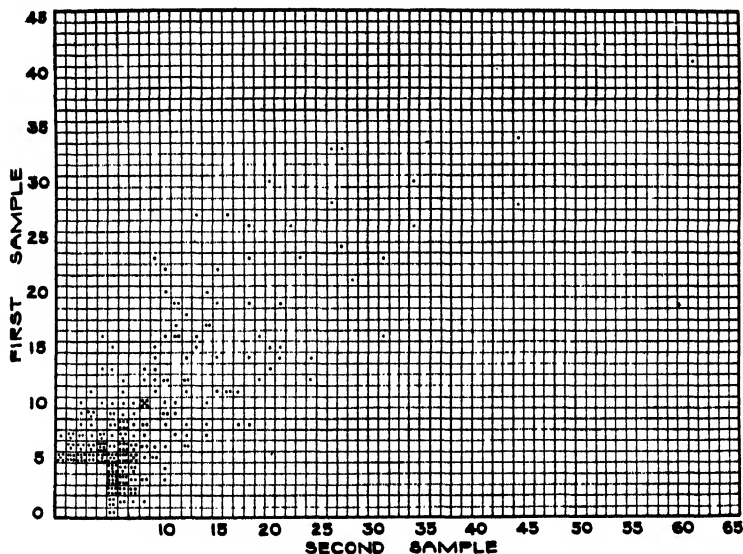


FIGURE 1.--The relation between the number of times an ancestor was found among the 800 random lines in the first 1926 sample and the number of times that the same ancestor was found in the 800 random lines of the second 1926 sample. Ancestors not found as often as five times in either sample have been admitted

CONCLUSION

These empirical tests of the method of Wright and McPhee lead to the conclusion that the method is about as accurate as its theoretical probable errors indicate, if all sources of systematic error which might prevent the lines from being truly random are carefully avoided. One must be continually on guard, however, against systematic errors which at first thought appear to be so trifling as not to be worth attention, but which under certain circumstances may creep into the supposedly random tracing of the lines.

OXIDATION-REDUCTION POTENTIALS AND THE HYDROGEN-ION CONCENTRATION OF A SOIL¹

By L. G. WILLIS

Soil Chemist, North Carolina Agricultural Experiment Station

INTRODUCTION

The oxidation-reduction equilibrium of soils has had scant attention from those interested in soil problems. There can be no doubt that it constitutes an important soil property, yet the publications of Gillespie² and of Remezow³ report the only formal investigations of the problem.

A number of observations made in the course of experimental work in eastern North Carolina pointed rather definitely to the conclusion that liming increased the reductiveness of those soils characterized as being naturally poorly drained, strongly acid, and high in organic matter. Some preliminary work showed that reproducible oxidation-reduction potentials could be obtained with one of these soils provided certain details of manipulation were followed. On the basis of this work a method was devised and the study of the effect of reaction on soil potential was undertaken.

EXPERIMENTAL DATA

EFFECT OF LIMING A SOIL ON THE OXIDATION-REDUCTION POTENTIAL

The soil selected for the work was a Dunbar fine sandy loam, one of a group of naturally poorly drained soils of the lower coastal plain in North Carolina. Five lots of this soil were limed at different rates and stored in 1-gallon glazed pots at 10 per cent moisture content for several months. They were then air-dried, the lumps were crushed, and samples for use were kept in glass-topped jars during the work.

Air-dry storage was adopted because a better-established equilibrium was maintained thereby, and also because it permitted greater facility in manipulation. Another possible advantage lay in the exposure of the soil to air while drying in order to oxidize as far as possible any reducing compounds formed by microbial action during incubation.

In preparation for the determination of the oxidation-reduction potential, 50 grams of the air-dry soil was shaken for three days in an unstoppered 250 c c volumetric flask of pyrex glass with 50 c c of distilled water in a constant-temperature air bath at 35° C. Erratic results obtained by shaking the sample at room temperature made it seem advisable to maintain a constant temperature during this operation.

¹ Received for publication Mar. 8, 1932; issued November, 1932. Contribution from the Agronomy Department, North Carolina Agricultural Experiment Station, as Paper No. 57 of the Journal Series.

² GILLESPIE, L. J. REDUCTION POTENTIALS OF BACTERIAL CULTURES AND OF WATER-LOGGED SOILS. *Soil Sci.* 9: 199-216, illus. 1920.

³ REMEZOW, N. P. DIE OXYDIERENDEN UND REDUZIERENDEN PROZESSE IN DEM PODSOLBODEN. *Ztschr. Pflanzenernähr., Düngung u. Bodenbk.* 15:34-44. 1929.

On the basis of the results shown in Table 1, the 3-day period of shaking the samples was adopted. The potentials recorded are from duplicate determinations and are calculated with reference to the normal hydrogen electrode.

TABLE 1.—*Oxidation-reduction potentials of soil-water suspension in duplicate determinations*

Period of shaking	Potentials	
	Volt	Volt
½ hour.....	+0.352	+0.383
1 day.....	.413	.409
2 days.....	.424	.435
3 days.....	.436	.436
4 days.....	.431	.436

The entire contents of the flask in which the samples were shaken were transferred to a specially constructed electrode flask illustrated in Figure 1.

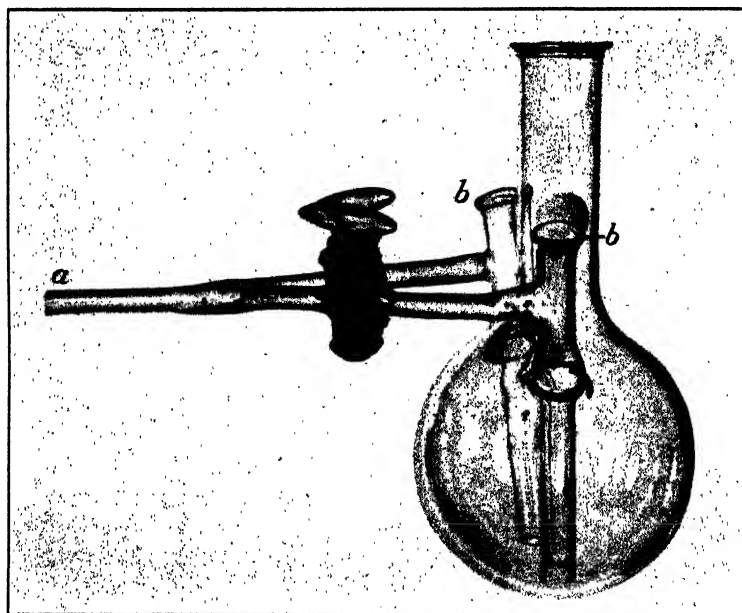


FIGURE 1.—Electrode flask for soils: a, Connection for nitrogen supply; b, electrode tubes

This was made by fusing two glass tubes into the side wall of a 300 cc round-bottom pyrex-glass flask in such a way that they reached nearly to the bottom inside the flask. Platinum foil electrodes about 5 mm square attached to glass tubing were inserted into these tubes and were held in place about 15 mm from the bottom by means of tightly fitting rubber stoppers at the tops of the tubes. The two tubes provided for duplicate readings from independent electrodes.

The electrode flask containing the soil-water mixture and with the electrodes in place was put into a controlled-temperature water bath at 35° C., and the air was withdrawn through the neck of the flask by means of an efficient vacuum pump, while the stopcocks leading into the electrode tubes were closed. After five minutes the stopcocks were opened enough to permit slow aspiration at reduced pressure with pure nitrogen gas. This was obtained by passing commercial tank nitrogen through an oxygen-absorption apparatus similar to the one described by Kendall.⁴

Aspiration was continued for 10 minutes, then the connection to the vacuum pump was closed and the electrode flask filled with nitrogen. The cocks admitting the nitrogen were then closed, and a partial vacuum was drawn, which was released with air at the neck of the electrode flask. This forced the soil-water suspension into the electrode tubes, covering the electrodes completely. The position of the electrodes in the tubes protected them from contact with oxygen either directly or by diffusion after the flask was opened.

After the electrodes had been immersed in the soil-water suspension 10 minutes a salt bridge from a saturated calomel cell was inserted through the neck of the flask to the soil suspension in the bottom and the potential difference read. Five minutes later another reading was taken.

There was usually a fair agreement between these readings, but since the change was usually toward a better conformity between the two electrodes at the second reading only the latter is reported. Successive readings over a period of several hours showed a slow drift toward a lower potential, but the differences between soils having different treatments were always approximately of the same order.

No attempt was made to test the necessity for removing the oxygen, which was done as an extra precaution to eliminate as far as possible all causes of erratic results. The potentials could hardly have been influenced by oxygen, as a calculation shows that the oxygen pressure as indicated by the potentials and pH values was approximately 10^{-30} atmospheres.

TABLE 2.—*The effect of liming on the oxidation-reduction potential of a soil*

Rate of liming in pounds per acre	Potential			Reaction
	Electrode A	Electrode B	Average	
	Volt	Volt	Volt	pH
None.....	+0.433	+0.433	+0.433	5.14
1,000.....	.415	.419	.417	6.10
2,000.....	.352	.344	.348	6.53
4,000.....	.302	.304	.303	7.65
6,000.....	.296	.299	.298	7.72
8,000.....	.296	.298	.297	7.75

The potentials given in Table 2 were read from duplicate electrodes in the same soil-water suspension. They are in virtual agreement with several other series run on the same samples in preliminary trials of the method.

Reactions were determined by means of the hydrogen electrode.

The evidence appears conclusive that liming decreased the oxidation-reduction potential of this soil. The effect is undoubtedly a

⁴ KENDALL, E. C. THE REMOVAL OF TRACES OF OXYGEN FROM NITROGEN. *Science* (n. s.) 73: 394-397, illus. 1931.

complex one, but there is some support in these results for the opinion that the lower potentials with the higher rates of liming are largely due to decreases in the hydrogen-ion concentration.

The evidence is not conclusive, however, because of the many possible effects of liming other than the modification of hydrogen-ion

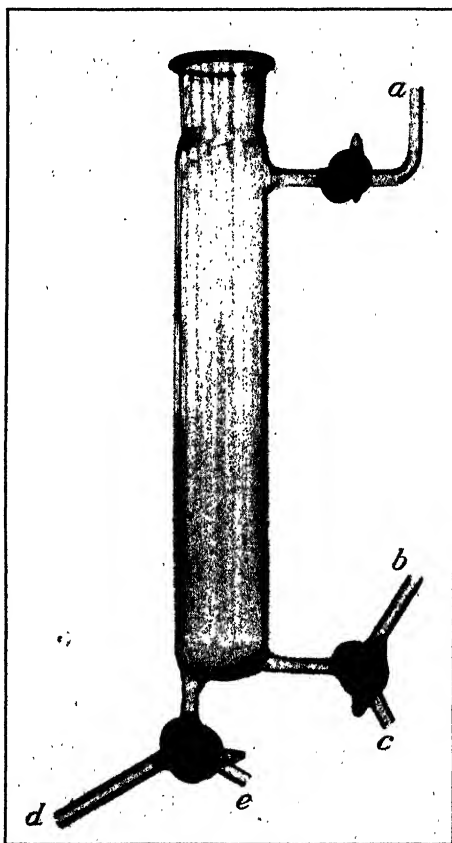


FIGURE 2.—Electrode tube for soil solution: *a*, Outlet to vacuum line; *b*, connection for nitrogen supply; *c*, outlet for withdrawing samples for pH determination; *d*, calomel-electrode contact; *e*, outlet for flushing salt-bridge contact

concentration. A closer approach to proof is found in some potentials obtained in a soil solution, the reaction of which was changed by titration with hydrochloric acid.

RELATION BETWEEN THE REACTION AND OXIDATION-REDUCTION POTENTIAL OF A SOIL SOLUTION

A sample of the soil used in the other experiments was mixed with calcium carbonate at the rate of 5 tons per acre and stored for several weeks at a moisture content of 15 per cent. This was then packed into an 8-liter glass percolater and the soil solution displaced with

water. About 150 c c of this solution was caught in the apparatus illustrated in Figure 2 from which the air had been displaced by nitrogen.

Oxygen was eliminated as before by reduced pressure and replacement with nitrogen, and potentials were read from duplicate electrodes placed in the apparatus near the bottom. Small portions of the solution were withdrawn for the determination of hydrogen-ion concentration, and then one-hundredth normal hydrochloric acid was added from a burette. The solution was well stirred by aspiration with nitrogen and the determinations repeated.

The results (fig. 3) show an inverse linear relation between oxidation-reduction potential and pH value.

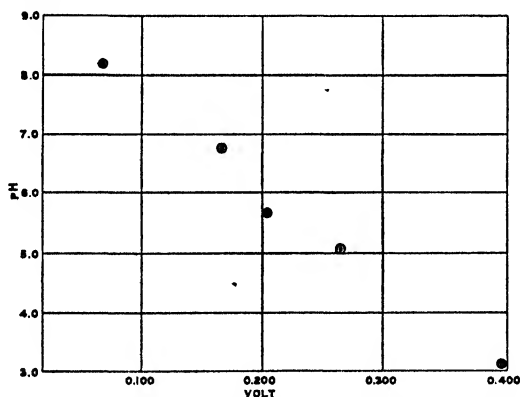


FIGURE 3.—Oxidation-reduction potentials of a soil solution at different pH values

DISCUSSION

The results reported in this paper are not in agreement with those of Remezow, who showed that limed soils had higher potentials than unlimed soils. Since the limed soils in the work he reported received applications of manure also and since the lime was applied several years prior to the determination of the potentials, his results are hardly comparable with those reported here.

Many applications of the data to recognized soil phenomena are suggested, but a discussion of these would necessarily be based on assumptions that are capable of proof.

SUMMARY AND CONCLUSIONS

The oxidation-reduction potential of a soil characterized as naturally poorly drained, strongly acid, and high in organic matter varies inversely with the pH values produced by liming.

A similar result is observed when the displaced solution from the limed soil is titrated with very dilute acid.

Within the category of soils where this relation between oxidation-reduction potential and hydrogen-ion concentration holds, rates of liming must be gauged with reference to the possible effects of the increase in reductiveness of the soil at the higher pH values.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 45

WASHINGTON, D. C., NOVEMBER 15, 1932

No. 10

QUALITY, SIZE, CAPACITY, GROSS ANATOMY, AND HISTOLOGY OF COW UDDERS IN RELATION TO MILK PRODUCTION¹

By W. W. SWETT, *Senior Dairy Husbandman*; FRED W. MILLER, *Senior Veterinarian and Physiologist*; and R. R. GRAVES, *Chief, Division of Dairy Cattle Breeding, Feeding, and Management, Bureau of Dairy Industry*; and G. T. CRECH, *Veterinarian, Pathological Division, Bureau of Animal Industry, United States Department of Agriculture*²

INTRODUCTION

The generally persistent belief among teachers and other professional men in dairy-cattle and veterinary work that the capacity for storing milk in the cow's udder is not more than about one-half pint to each quarter, and that the greater part of the milk obtained at any milking is secreted during the milking process, has been discussed in a previous publication.³ Evidence has been presented which shows beyond question that a very large proportion, if not all, of the milk obtained at any milking is stored within the udder before the milking process is commenced.⁴

The purpose of this paper is to discuss the quality, size, and general characteristics of the udders used in post-mortem milking studies; to describe and illustrate their gross and microscopic anatomy; to show that not only is the milk stored within the udder, but that the capacity of the secretory system is sufficient to hold considerably more than the quantity produced at a milking; and to show the relation of udder characteristics to the demonstrated producing ability of the cow.

The regular procedure adopted for studying the mammary gland in relation to its producing capacity is to examine it frequently and regularly through its different stages of development and activity, to determine its size (empty weight), to measure the capacity of its secretory system after amputation, and to study the gross and microscopic structure and the physical properties of its tissue.

METHODS OF STUDY

JUDGING THE CHARACTERISTICS OF THE UDDER BY ANTE-MORTEM EXAMINATION

Ante-mortem examinations to determine the comparative type, quality, and general characteristics of udders are made on all cows in the breeding herd of the Bureau of Dairy Industry at Beltsville, Md. Observations of this character have been made on more than 250 cows.

¹ Received for publication Feb. 4, 1932; issued November, 1932.

² The histological study of the udders on which this report is based was made possible through the courtesy of J. R. Mohler, Chief of the Bureau of Animal Industry, who made available the personnel and facilities of the Pathological Division for the conduct of that phase of the work.

³ SWETT, W. W. RELATION OF THE CONFORMATION AND ANATOMY OF THE DAIRY COW TO HER MILK AND BUTTERFAT PRODUCING CAPACITY. UDDER CAPACITY AND MILK SECRETION. *Jour. Dairy Sci.* 10: 1-14. 1927.

⁴ ———. *Op. cit.*

——— MILLER, F. W., and GRAVES, R. R. QUANTITY OF MILK OBTAINED FROM AMPUTATED COW UDDERS. *Jour. Agr. Research* 45: 385-400, illus. 1932.

In most cases monthly observations have been made through at least one lactation period. The condition of the udder is determined by visual examination, by manipulation, and by palpation. More than 30 items are recorded at each examination. Only a few of the items which may be significant in relation to "quality of udder" are discussed in this paper. These are (1) looseness of udder, (2) "yieldability" of udder, (3) softness and mellowness of gland tissue, (4) abundance of fiber, and (5) average free space.

The looseness of the udder is the extent to which its covering can be stretched. The "yieldability" of the udder is the degree to which it responds to pressure. The softness and mellowness of the gland tissue is the extent to which it responds to kneading, and is judged entirely by palpation. The abundance of fiber present in the gland tissue also is determined by palpation. Gland tissue which is rough and harsh and appears to be corrugated on the surface is classed as fibrous. That which feels smooth and of uniform consistency is classed as not fibrous. The free space above each teat is the distance above the base of the teat which can readily be compressed without marked resistance. This is determined for each quarter, but since a considerable variation is found in the different quarters of the same udder, the average is used as an index of free space for the udder.

It is recognized that in any grading system based on judgment, the personal element can not be entirely avoided. The use of such a system, however, is necessary in dealing with factors that can not be definitely measured, and is considered satisfactory for this study.

Looseness and yieldability of the udder and softness and mellowness of the gland tissue are generally accepted as indicative of good udder quality, and therefore are considered desirable. An abundance of fiber within the gland tissue of an udder is not ordinarily considered desirable, but does not necessarily indicate lack of quality. The amount of free space above the teat in each quarter might be considered as indicative of the size and character of the cistern. The amount of free space may be associated with udder quality because it is almost certain to be relatively greater in a loose, soft, and mellow udder than in a tight and hard one.

In evaluating the observations of udder quality, a grading system is used for each of the quality items, the grades ranging from a maximum of 9 to a minimum of 1. In each case a grade of 9 indicates the maximum of the characteristic under consideration, but does not necessarily signify excellence. For example, in the case of degree of fiber within the gland tissue, a grade of 9 would indicate extreme abundance of fiber regardless of whether a fibrous condition is desirable or objectionable. To illustrate the application of this grading system to characteristics ordinarily described in words, the nine grades for looseness and their word equivalents are given as follows: 9, extremely loose; 8, very loose; 7, loose; 6, medium to loose; 5, medium; 4, medium to tight; 3, tight; 2, very tight; and 1, extremely tight. The same grades can be applied to any of the items under consideration. As the free space can be measured in definite units, it is first recorded in inches. In order to permit the data for free space to be combined with grades for other items representing quality, the measurements of free space are converted into similar grades ranging between 1 and 9. Both inches and grades are given.

A combination of the grades for each of the five quality items is referred to as the composite grade for quality.

Ordinarily ante-mortem observations are made both before and after milking. The after-milking grades are used whenever they differ from the premilking grades. In all but three of the cases reported in this paper the ante-mortem observations were made within 16 days of the time of slaughter. The udder of cow 459 was examined 27 days before slaughter. At the time of slaughter cow 123 was just finishing the seventh month of a lactation period. The latest previous examination of her udder for quality was made late in the previous lactation period and is considered satisfactory for use. The latest examination of cow 272 was made when she had just finished the twelfth month of her first lactation and was nearly dry. She was not killed until 651 days later, when she was in an earlier stage of her third lactation and was producing 20 to 24 pounds of milk on one milking daily. Because of the difference in age, stage of lactation, and length of time between observation and slaughter, the observation grades for this cow are omitted.

The external form and general characteristics of the 11 udders as they appeared before the death of the cows are illustrated in Plate 1.

POST-MORTEM STUDIES OF THE AMPUTATED UDDER

In studying the size, capacity, and anatomy of the mammary gland in relation to its ante-mortem characteristics and its producing capacity, the udder is amputated immediately after the cow is killed and bled. After amputation the udder is attached to a frame in as nearly as possible a natural position and suspended by its surrounding skin and the median septum. If post-mortem milking tests are conducted they take place at this time. The empty weight of the amputated udder is then determined either by direct weighing or, when post-mortem milking tests are conducted, by deducting the weight of its contents from the total weight of the udder and contents. The secretory system of half of the udder is then filled by injecting 10 per cent formalin through the teats, the amount of fluid retained being used as a measure of the capacity of the secretory system of that half. After the half is filled the udder is divided into halves by cutting along the median septum. The filled half is frozen, cut with a saw into vertical transverse sections approximately 1 inch thick, and each section is photographed to furnish a permanent record of the gross anatomy of the gland at regularly spaced planes from its anterior to its posterior extremity. Blocks of tissue are taken from definitely located areas of both quarters of the untreated half for histological study.

RECOVERY OF MILK FROM THE AMPUTATED UDDER

The percentage of post-mortem recovery, or proportion of ante-mortem yield of milk obtained by milking the amputated udder, has been determined for each of the 11 cows discussed in this paper. The method employed in arriving at the percentage of post-mortem recovery was to determine over a period of 10 days the ante-mortem milking level of the cow. During this period the cow was milked, fed, and watered at a definite hour and every effort was made to maintain uniformity in her management. Slaughter took place on the eleventh

day, at the hour on which milking had taken place on previous days. As soon as the cow was stunned and bled the udder was amputated, suspended from a suitable frame in a natural position, and milked out by hand in the usual manner. Four hours later a second post-mortem milking took place for the purpose of removing the milk which had meanwhile drained into the cisterns and lower ducts. By dividing the total number of pounds of milk obtained in the first and second post-mortem milkings by the average number of pounds of milk obtained at corresponding ante-mortem milkings, the percentage of post-mortem recovery was obtained.

SIZE (EMPTY WEIGHT) OF UDDER

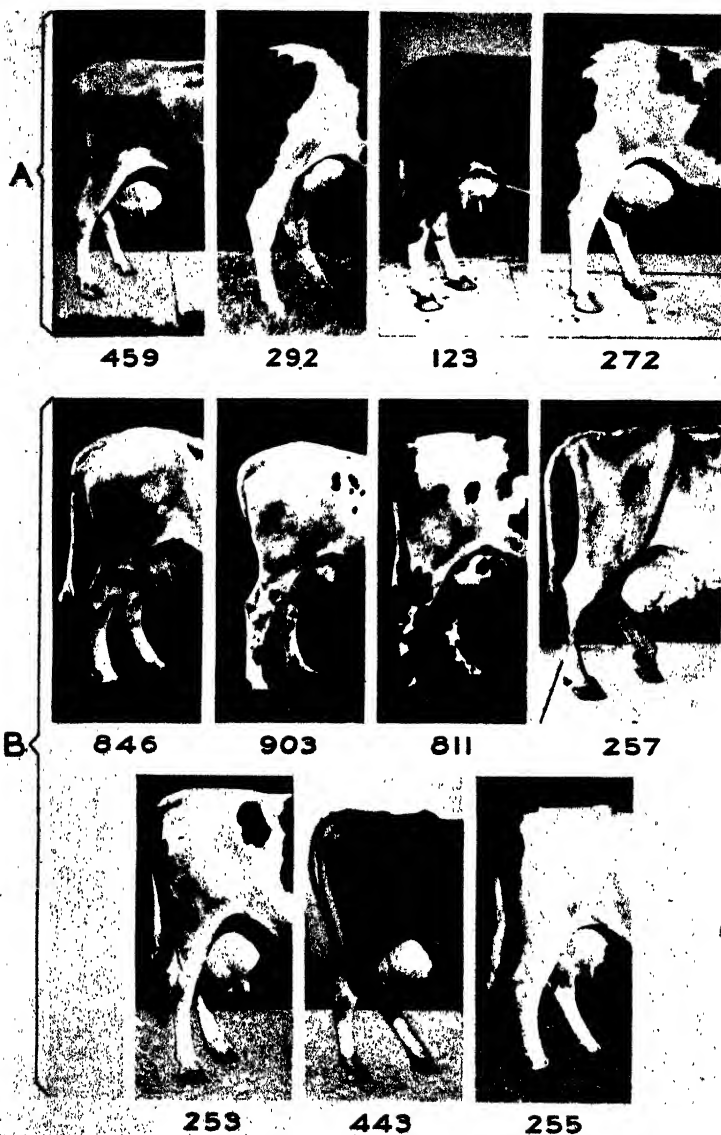
Size of udder is very difficult to determine in the living cow. Udders vary greatly in shape, and the definite landmarks necessary for measuring udder dimensions are not readily established. Furthermore, a determination of udder volume in the living cow by displacement would be attended with many difficulties. For example, it would be nearly impossible to make any equitable allowance for the degree of concavity of the upper surface of the udder, which is affected by variations in the inaccessible contour of the abdominal wall within the boundaries of the udder attachment. In these experiments each cow was slaughtered before being milked. The difference between the weight of the amputated udder in pounds before the post-mortem milking was performed and the total number of pounds of milk obtained at the first and second post-mortem milkings was used to represent the size or empty weight of the udder.

The retention of small quantities of milk, which in some cases was indicated after the second post-mortem milking, conceivably would introduce an error in the empty weight of udder as determined in this manner. Such an error in actual practice, however, was probably relatively slight and appears to have been unavoidable.

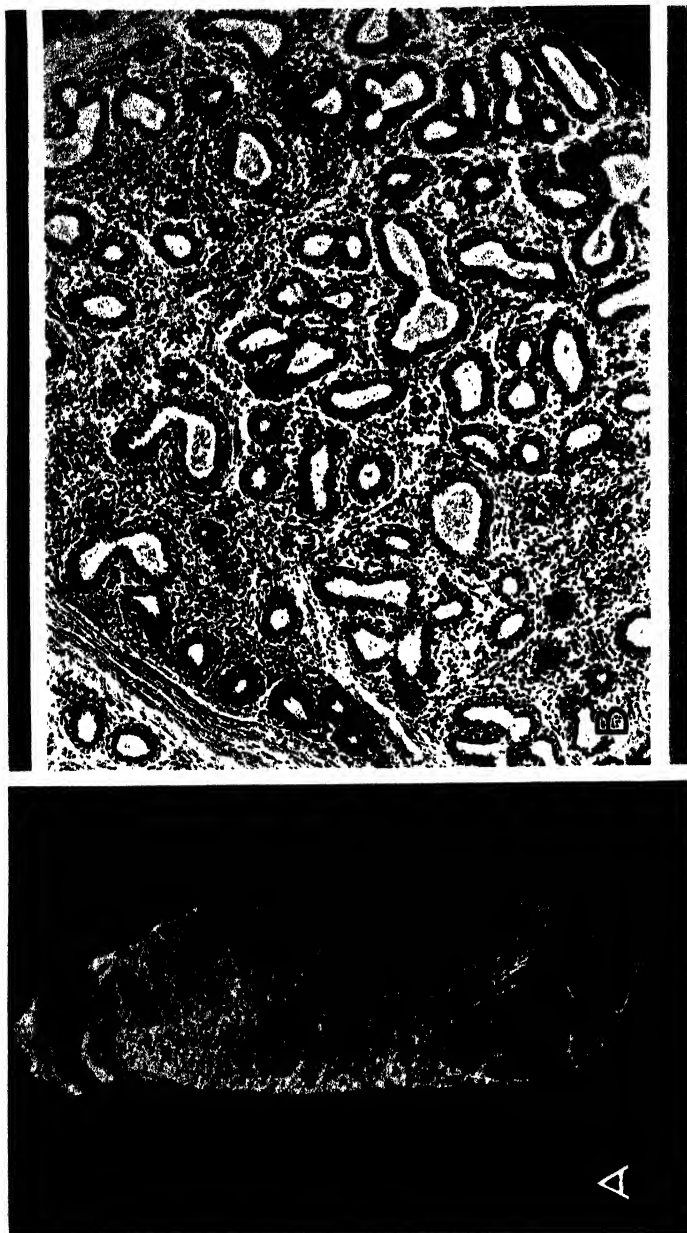
CAPACITY OF UDDER

As previously stated, the secretory system of half of the udder (usually the right half) was filled by injecting 10 per cent formalin through the teats, the quantity of formalin retained within this half of the udder being measured. Twice the quantity of formalin retained in the half of the udder filled in this manner gives the calculated volume capacity of both halves. This total volume in cubic centimeters is divided by 1,000 and multiplied by 2.2725 to give the "milk-equivalent capacity of the udder," or the weight in pounds of an equal volume of milk of average specific gravity. In the discussions which follow, "udder capacity" or "capacity of udder" refers to "milk-equivalent capacity of the udder," determined in this manner.

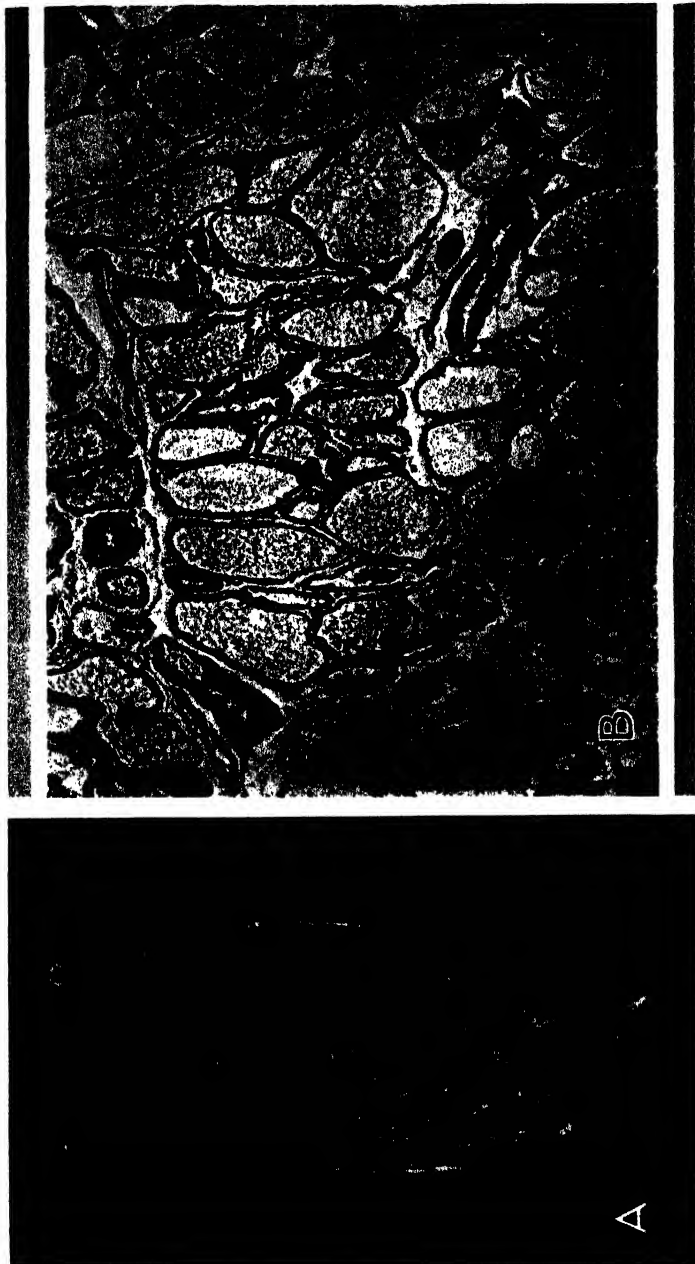
In the first 10 cases here reported, the formalin was injected by pressure obtained with a hand pump, but in filling the first 5 of these amputated udders the amount of pressure was not recorded. A gage was then attached to the pump to show the pressure at the point of injection (the teat). Accurate determination of the pressure by this method was difficult, for it might be 15 to 30 pounds on the stroke of the pump but would diminish as soon as the stroke was completed. The stability of pressure after cessation of pumping varied consider-



External appearance of the 11 udders included in this study. A, Group 1; B, group 2



A, Vertical transverse section through a rear quarter of udder 459; B, photomicrograph of tissue from udder 459



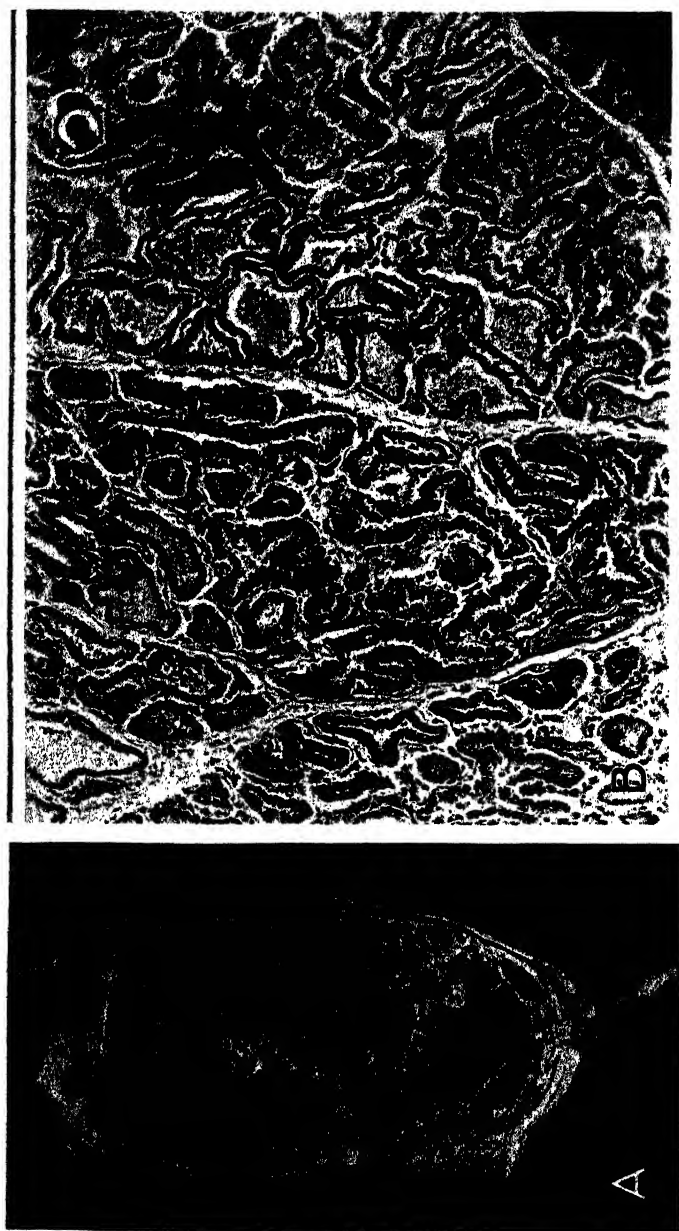
A, Vertical transverse section through a rear quarter of udder 292; B, photomicrograph of tissue from udder 292



A, Vertical transverse section through a rear quarter of udder 123; B and C, photomicrographs of tissue from udder 123—B, from normal-functioning quarter; C, from "blind" quarter



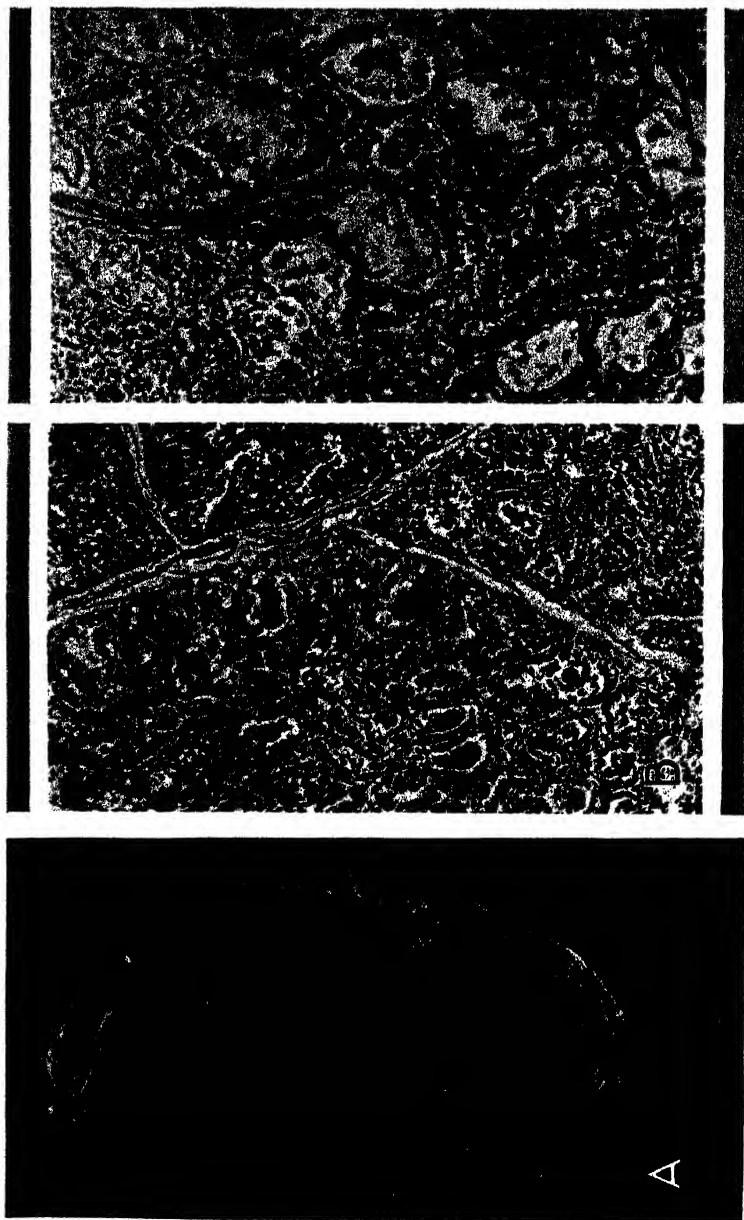
A, Vertical transverse section through a rear quarter of udder 272, B, photomicrograph of tissue from udder 272



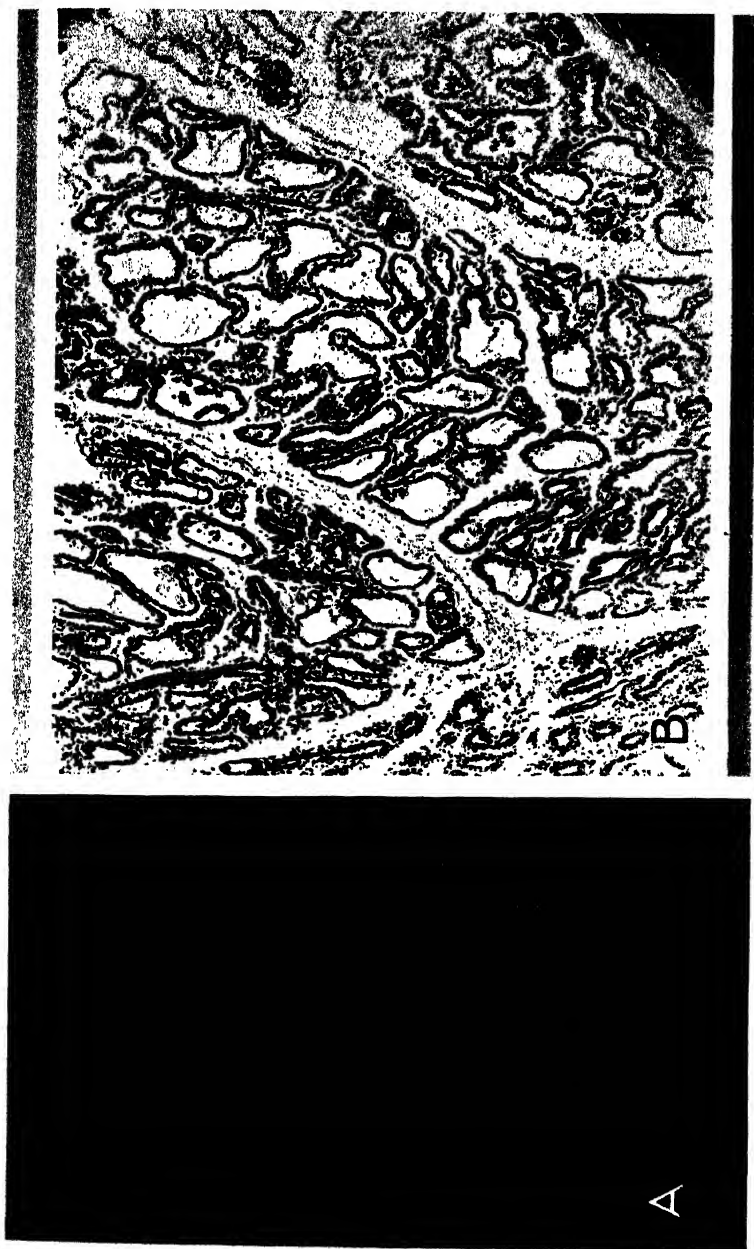
A, Vertical transverse section through a rear quarter of udder 846; B, photomicrograph of tissue from udder 846



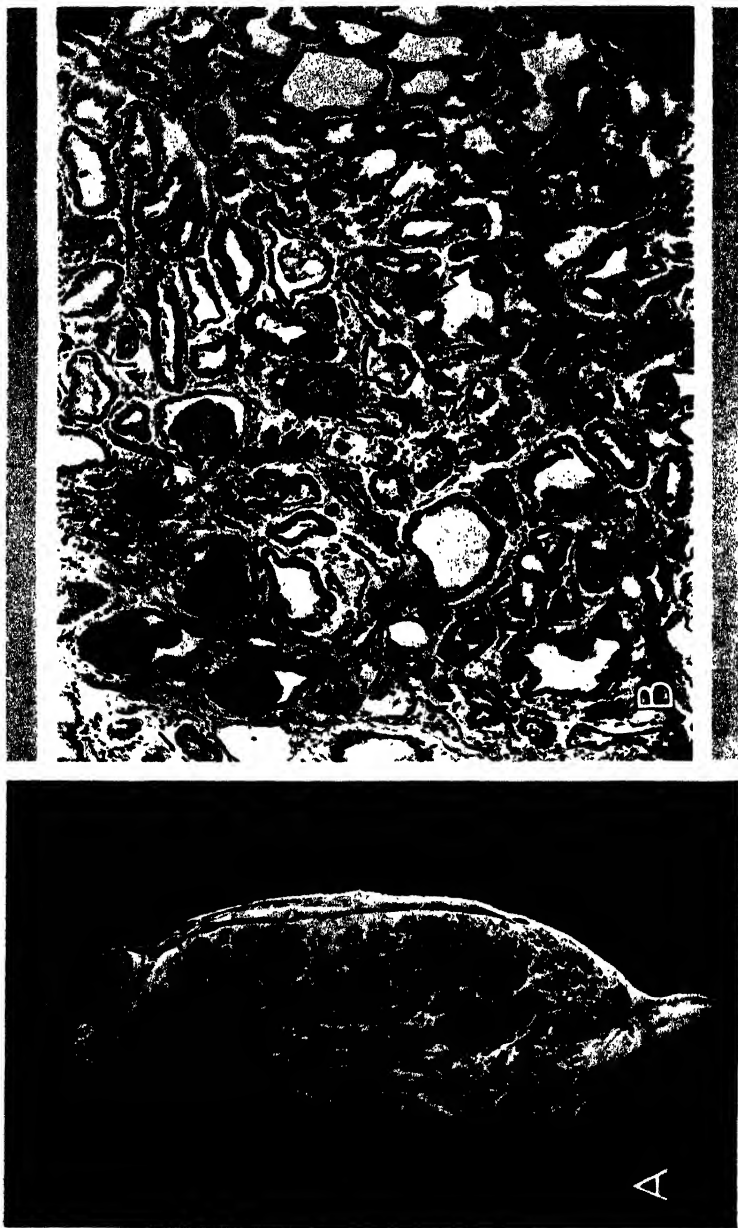
A, Vertical transverse section through a rear quarter of udder 903; B, photomicrograph of tissue from udder 903



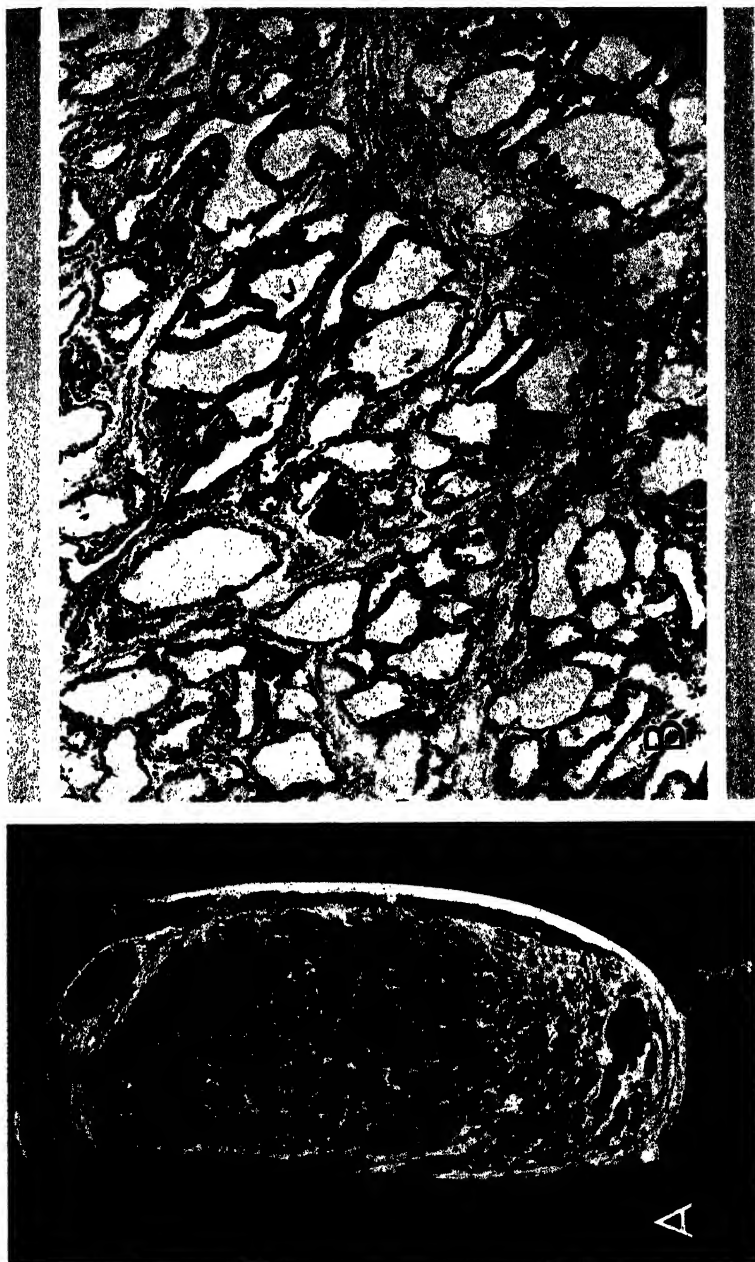
A, Vertical transverse section through a rear quarter of udder 811; B and C, photomicrographs of tissue from udder 811—B, from undersized quarter producing relatively small quantity of milk; C, from quarter recently affected by garget



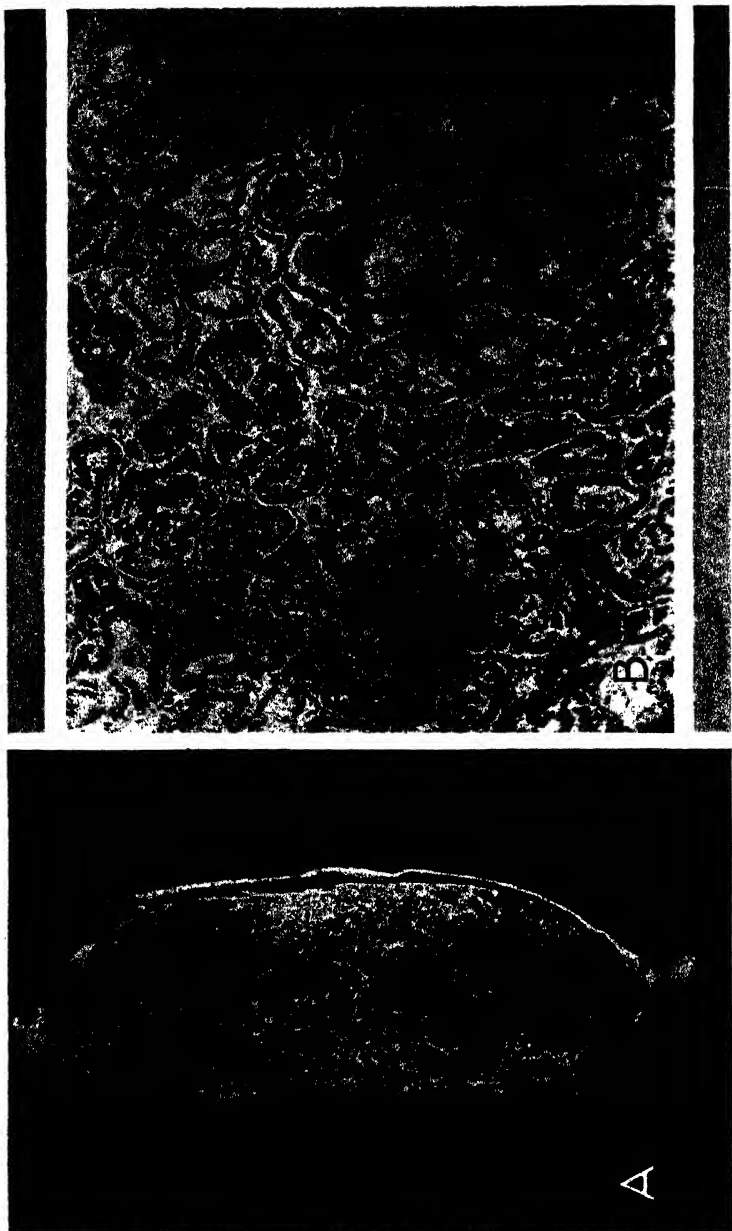
A, Vertical transverse section through a rear quarter of udder 257; B, photomicrograph of tissue from udder 257



A. Vertical transverse section through a rear quarter of udder 253; B. photomicrograph of tissue from udder 253



A, Vertical transverse section through a rear quarter of udder 443; B, photomicrograph of tissue from udder 443



A, Vertical transverse section through a rear quarter of udder 255; B, photomicrograph of tissue from udder 255

ably with different udders. Pumping was continued until the udder was firm and well distended and the pressure had become fairly steady. For the next 5 udders the pressure became reasonably stable at $3\frac{1}{2}$ to 6 pounds, or at an average of about 5 pounds. Later a tank was installed at an elevation sufficient to produce a gravity pressure of 10 pounds at the level of the udder, making it possible to obtain a constant pressure. About five minutes was required to fill an udder. The eleventh udder was filled by gravity pressure in the manner described.

RELATION OF CAPACITY TO WEIGHT OF UDDER

The "relation of capacity to weight of udder" expressed as a percentage is obtained by dividing the udder capacity by the weight of the empty udder and multiplying the result by 100. This relationship indicates the fluid-holding capacity of the secretory system for each 100 pounds of udder weight, and in a sense signifies the relative openness, porosity, or sponginess of the udder tissue.

GROSS ANATOMY

The gross anatomy of the cow's udder has been known in a general way for many years. Little is known, however, of the relation of the gross anatomy to the individual development, quality, and general characteristics of the udder, and the maximum producing capacity of the cow. A comparative study of this nature has been undertaken in the experiment-station herd of the Bureau of Dairy Industry at Beltsville, Md. Partial results are reported in connection with the 11 cows on which this report is based. The method of preparing and photographing consecutive vertical transverse sections from the extreme rear to the extreme front of one half of the udder has previously been outlined.⁵ Plates 2 to 12, inclusive, show for each of the 11 lactating udders the gross anatomy of a section through the rear teat. A brief description of the gross structure of a rear quarter of each amputated udder appears in the discussion of the udder characteristics, life history, and performance of the individual cows.⁶

Great variations in type are shown in the illustrations of gross structure. Some of the udders have large, distinctly circumscribed and undivided cisterns, whereas others have very small cisterns which may be distinct in outline or which may consist only of a network of small communicating cavities. In some of the udders the tissue appears to be firm and dense; in others it is open and porous and the large ducts are distributed over a large proportion of the surface of the sections. Some of the udders have large quantities of connective tissue, appearing in white streaks; others have comparatively little.

Measuring any one of these three variables—relative size of cistern, openness of tissue, and proportion of connective tissue—in definite units would be particularly desirable, but obviously is difficult if not impossible. Some means of grading the udders or of comparing them with each other on the basis of these three variables is desirable in order that they may be correlated with udder quality characters, post-mortem recovery of milk, size (empty weight) of udder, udder capacity, and milk-production records.

⁵ Report of the Chief of the Bureau of Dairy Industry, U. S. Dept. of Agr., Dec. 11, 1929, p. 8.

⁶ The age and stage of lactation of each cow studied are given in Table 1.

In the absence of a satisfactory method of recording these characteristics in definite units of measure, the 9-unit grading system previously described was used. Four men working independently assigned grades to each of the three variables, and a composite of the four individual ratings so obtained for each variable was prepared for use as a basis for determining the previously mentioned relationships between gross anatomy and the quality, capacity, and performance of the udder. Remarkable closeness in agreement was shown by those who graded the gross anatomy variables.

In addition to the grading of the gross-anatomy characteristics just described, an attempt was made to measure with a planimeter the size of the cistern. Photographs of two or more sections through a rear quarter of the udder were selected as representative. The photographs were the same as those used in assigning grades to the gross-structure characteristics. The cistern was measured as accurately as possible, then the area of the entire section was determined and the ratio of the former to the latter was calculated. On account of the very great variation in type and shape of cisterns, measurement of their areas was usually extremely difficult. Measurement of the distinctly circumscribed cistern, however, was comparatively simple even though its surfaces tapered so greatly that the areas at the two cut surfaces of a single section were in many cases greatly different from each other. Measurement of the divided cisterns, and in some cases this condition was extreme, was almost impossible. The measured and graded values checked very closely for the larger and more open cisterns. In general, however, those udders having divided or porous cisterns received a higher rating when graded visually than when measured, because during the visual examination allowance was automatically made for the area of the small cavities making up the cistern. On account of the indefiniteness of the shape and boundaries of these cavities, however, a measurement of many of them proved hardly feasible and was omitted.

HISTOLOGY ⁷

Although the general microscopic structure of the mammary gland has long been known, definite knowledge of the comparative histology of the udders of different cows, or the relation of histology of the udder to its individual characteristics and producing capacity is almost entirely lacking. A brief description of the histology of each of the 11 udders is presented in the discussion of udder characteristics, life history, and performance of the individual cows. In each case the discussion is based on a study of tissue selected from several definitely located areas in the half of the udder not treated with formalin. Plates 2 to 12, inclusive, show the histological appearance of the tissue from each of the 11 udders included in this study.⁸

PRESENTATION OF RESULTS

For the convenience of the reader the data showing the percentage of the ante-mortem production obtained by post-mortem milking⁹ are repeated in Table 1, which also gives grades for quality, size

⁷ The histological studies of the 11 udders were made by G. T. Creech.

⁸ The age and stage of lactation of each cow studied are given in Table 1.

⁹ SWETT, W. W., MILLER, F. W., and GRAVES, R. R. Op. cit.

(empty weight), capacity of secretory system, relation of capacity to weight, and gross anatomy ratings for the 11 udders included in the study. The first four cows constitute group 1. The next seven, which represent more carefully controlled conditions before and at the time of slaughter, and maintenance of body temperature in the amputated udders, are assembled in group 2.

SIGNIFICANCE OF UDDER QUALITY

To show the relationships which may exist between the quality grades assigned to udders on ante-mortem examination, and the characteristics of the same udders as determined after amputation, a number of coefficients of correlation¹⁰ have been determined between the six udder-quality items given in Table 1 and the post-mortem recovery of milk, the size (empty weight) of udder, the capacity of udder, and the relation of capacity to weight of udder. It is unfortunate that data from a greater number of animals are not available for analysis in this study.

The degree of relationship between the individual udder-quality items and the other udder characteristics is shown by the magnitude of the simple correlations. A greater number of observations, of course, would have made possible a determination of the relationships with greater accuracy. The relation between any one udder-quality item and the post-mortem recovery of milk, for example, might be affected by the influence of any or all of the other udder-quality items. In order to make this study in the detail desired, net relationships by statistical methods would have to be determined, which would require establishing the extent of existing correlation for each pair of items, holding all other items constant. This has not been done, as the data available for this study are not sufficient to justify it.

RECOVERY OF MILK FROM AMPUTATED UDDERS

The degree of relationship between the ante-mortem udder-quality items listed in Table 1 and the post-mortem recovery of milk from the amputated udders is indicated by the correlation coefficients which follow:

Looseness of udder.....	+ 0. 7586 ± 0. 0905
Yieldability of udder.....	+ . 7964 ± . 0780
Softness and mellowness of gland tissue.....	+ . 4003 ± . 1781
Abundance of fiber.....	+ . 5837 ± . 1406
Average free space (inches).....	+ . 6089 ± . 1342
Composite grade for quality.....	+ . 8451 ± . 0610

Every coefficient of correlation in this group is positive, and with one exception (softness and mellowness of gland tissue) every one is significant. It appears, therefore, that a definite relationship exists between the quality of the udder as judged on ante-mortem examination and the post-mortem recovery of milk. It seems reasonable to suppose that milk could be drawn more completely and more quickly from an udder of good quality than from one of poor quality.

¹⁰ Throughout this paper, in presenting each coefficient of correlation a straight line relationship is assumed between the two variables.

TABLE 1.—Udder quality of 11 cows and the relative post-mortem recovery of milk, size (empty weight), capacity, and gross anatomy of their amputated udders

Cow No.	Breed of cow	Stage of lactation at time of—		Quality of udder as judged by ante-mortem examination							Milk obtained from udder						
		Ante-mortem examination	Slaughter	Age at time of slaughter	Looseness of udder	Yieldability of udder	Softness and melowness of gland tissue	Abundance of fiber	Average free space	Composite of grades for quality	Ante-mortem	Post-mortem	Per cent of ante-mortem recovered post-mortem				
Group 1	Jersey	Months	Days	Months	Days	Years	Months	Grade	Grade	Grade	Inches	Grade	Pounds	Pounds	86.09		
	459	2	28	2	8	8	6	9	6	7	5.00	8	12.07	10.27	49.58		
	322	1	12	1	12	2	3	5	6	4	2.75	4	21.38	10.60	69.43		
	123	12	11	6	28	4	3	8	7	7	2.83	4	10.87	7.20	40.31		
	272			6	25	4	10						21.83	8.90			
Average													16.41	9.22	61.10		
Group 2	Grade Holstein	3	25	4	1	6	2	9	9	9	5.00	8	18.51	18.70	101.03		
	do	13	27	14	8	5	2	8	7	7	4.81	37	16.27	11.60	71.30		
	do	23	16	24	1	6	9	7	7	5	4.25	33	13.20	9.70	63.82		
	Holstein	1	11	1	22	10	9	8	5	6	2.00	36	21.22	15.35	62.07		
	do	13	20	14	0	9	4	8	8	8	3.63	34	21.25	15.35	72.71		
	Jersey	6	8	6	19	9	4	8	6	6	3.31	35	17.41	15.20	87.31		
	255	0	24	1	0	9	7	8	7	7	3.88	6	25.80	17.90	68.99		
Average													19.88	14.83	75.32		
Average of 2 groups								7.9	7.1	6.2	6.7	3.75	5.8	33.7	18.62	12.79	70.15

Cow No.	Breed of cow	Capacity of udder				Gross anatomy					
		Size (empty weight) of amputated udder	Portion of udder filled		Quantity of fluid held by portion filled	Milk equivalent	Relation of capacity to weight of udder	Relative size of cistern		Proportion of connective tissue	
			Pounds	Quarters				Graded	Measured		
											Cubic centimeters
Jersey	27.23	2 right	6.200	28.18	103.49	Grade	27.0	9.41	Grade	12	
292	Holstein	44.40	2 right	10.200	46.36	104.41		17.0	3.81		6
123	Guernsey	23.30	2 left	7.660	34.81	149.47		25.5	7.45		10
272	Holstein	46.70	2 right	10.660	48.45	103.75		13.0	2.53		11
	Average	35.41		8.680	39.45	115.26					
Group 2											
846	Grade Holstein	25.80	2 right	13.800	62.72	243.10		24.0	6.42		6
903	do.	36.90	2 right	14.390	65.40	177.24		9.0	6.66		16
811	do.	32.30	2 right	13.840	62.90	194.75		17.5	3.21		8
257	Holstein	54.15	2 left	12.030	54.68	100.98		5.5	2.10		9
253	do.	44.80	2 right	11.880	54.00	120.54		13.5	5.59		20
443	Jersey	27.55	2 right	8.330	37.86	137.42		9.0	8.80		20
255	Holstein	42.70	2 right	12.300	55.90	130.91		17.0	2.13		17
	Average	37.74		12.367	56.21	157.85					
	Average of 2 groups	36.89		11.026	50.11	142.36		16.2	4.03		12.3

SIZE (EMPTY WEIGHT) OF UDDER

The degree of relationship between each of the ante-mortem udder-quality items, and the size (empty weight) of udder is shown by the following correlation coefficients:

Looseness of udder.....	-0. 2868 ± 0. 1957
Yieldability of udder.....	- . 6353 ± . 1272
Softness and mellowness of gland tissue.....	- . 5397 ± . 1512
Abundance of fiber.....	- . 3553 ± . 1884
Average free space (inches).....	- . 5180 ± . 1561
Composite grade for quality.....	- . 6565 ± . 1214

In contrast to the coefficients of correlation for post-mortem recovery of milk from the amputated udders, of which all are positive and all but one significant, those for size of udder are all negative, indicating an inverse relationship, and only two (yieldability of udder and composite grade for quality) are definitely significant. Two others (softness and mellowness of gland tissue and average free space) are of moderate or doubtful significance. Looseness of udder and abundance of fiber appear to have no relation to size of udder. A negative or inverse relationship between quality and size of udder is indicated.

CAPACITY OF UDDER

The degree of relationship between each of the udder-quality items and capacity of udder is shown by the following correlation coefficients:

Looseness of udder.....	-0. 0488 ± 0. 2128
Yieldability of udder.....	+ . 0882 ± . 2116
Softness and mellowness of gland tissue.....	+ . 0544 ± . 2127
Abundance of fiber.....	+ . 0899 ± . 2116
Average free space (inches).....	+ . 2198 ± . 2030
Composite grade for quality.....	+ . 1473 ± . 2087

In this group one coefficient is negative and five are positive, but the probable error is greater than the coefficient, except in one case where they are nearly equal. None of the correlation coefficients in this group is of any significance whatever. Apparently quality and capacity were not in any way related in this group of udders.

RELATION OF CAPACITY TO WEIGHT OF UDDER

The degree of relationship between each of the ante-mortem udder-quality items and "relation of capacity to weight of udder", which is an index of its relative fluid-holding capacity or its porosity, is indicated by the following correlation coefficients:

Looseness of udder.....	+ 0. 2112 ± 0. 2038
Yieldability of udder.....	+ . 6356 ± . 1271
Softness and mellowness of gland tissue.....	+ . 5591 ± . 1466
Abundance of fiber.....	+ . 3284 ± . 1903
Average free space (inches).....	+ . 5748 ± . 1428
Composite grade for quality.....	+ . 6767 ± . 1156

Every one of the coefficients in this group is positive. Three of them (yieldability of udder, average free space, and composite grade for quality) are distinctly significant, and another (softness and mellowness of gland tissue) might be classed as significant. The other two (looseness of udder and abundance of fiber) are not signifi-

cant. The similarity between the coefficients in this group and those for size (empty weight) of udder is striking, although all of those for relation of capacity to weight of udder are positive, whereas all of those for size of udder are negative. It appears that a number of the udder quality characteristics are positively correlated with relation of capacity to weight of udder.

In the four separate considerations of udder quality in relation to other udder characteristics, distinctly or moderately significant correlation coefficients were obtained for each of the quality items the following number of times: Looseness of udder, once; yieldability of udder, three times; softness and mellowness of gland tissue, twice; abundance of fiber, once; average free space, three times; and composite grade for quality, three times. The relation of quality grades in general to the percentage of post-mortem recovery of milk is the most significant, and their relation to the size of udder and to the relation of capacity to weight of udder are similar and relatively significant, but their relation to capacity of udder is of no significance.

FACTORS AFFECTING RECOVERY OF MILK FROM AMPUTATED UDDERS

The possible effect of management and method of killing the cows and the condition under which the amputated udders were kept, on the post-mortem recovery of milk, has been discussed in a previous paper.¹¹ The quantity of milk obtained from the amputated udders ranged from 7.20 to 18.70 pounds. The lowest and highest post-mortem recoveries were, respectively, 40.31 per cent and 101.03 per cent of the quantity obtained at corresponding ante-mortem milkings. (Table 1.) The degree of correlation between post-mortem recovery and ante-mortem udder quality grades is shown in a previous paragraph to be high. The coefficients showing the degree of correlation between the post-mortem recovery of milk and the size (empty weight) of udder, the capacity of udder, and the relation of capacity to weight of udder, follow:

Size (empty weight) of udder.....	--0. 6750 ± 0. 1107
Capacity of udder.....	- . 0516 ± . 2028
Relation of capacity to weight of udder.....	+ . 5611 ± . 1393

The highest post-mortem recovery of milk (101.03 per cent) was obtained from the second lightest udder, the lowest post-mortem recovery (40.31 per cent) was obtained from the second heaviest udder, the second and third highest post-mortem recoveries were obtained from udders well below the average weight of udders included in this study, and the second and third lowest post-mortem recoveries were obtained from udders well above the average in weight. The udder of cow 903 weighed 36.90 pounds, which is almost the exact average weight for the entire group, and yielded 71.30 per cent, which is close to the average post-mortem recovery (70.15 per cent). In this group of udders, size is negatively or inversely correlated with post-mortem recovery, indicating that the small udder of good quality milks out most readily after amputation. No correlation was found to exist between capacity of udder and the readiness with which it released its milk post-mortem. The relation of capacity to weight of udder, however, appears to be positively

¹¹ SWETT, W. W., MILLER, F. W., and GRAVES, R. R. Op. cit.

correlated with post-mortem recovery or milking-out efficiency. A close relationship between quality of udder and relation of capacity to weight of udder is again suggested.

In considering the possible effect of the milking level immediately before slaughter on the recovery of milk from the udders after amputation, one might logically anticipate a lower percentage of post-mortem recovery from an udder producing at a high level than from one producing at a lower level just before slaughter. The ante-mortem production at a milking for the 11 cows studied ranged from 10.37 to 25.80 pounds and averaged 18.62 pounds. The cow having the lowest ante-mortem level (10.37 pounds) yielded 69.43 per cent on post-mortem milking. The one having the highest ante-mortem milking level (25.80 pounds) yielded almost the same proportion (68.99 per cent) on post-mortem milking. The sixth or middle cow in the group, from the standpoint of ante-mortem lactating level, whose ante-mortem production (18.51 pounds) was very close to the average for all the cows, yielded 101.03 per cent, which was by far the highest post-mortem recovery recorded for any cow. These facts might indicate little if any relation between ante-mortem producing level and post-mortem recovery. On the other hand, the second and third highest post-mortem recoveries (87.31 and 85.09 per cent) were from udders whose ante-mortem producing levels were below the average, and the two lowest post-mortem recoveries (40.31 and 49.58 per cent) were from udders whose ante-mortem producing levels were well above the average. The coefficient of correlation between the ante-mortem producing level of the 11 udders immediately before slaughter and the percentage of post-mortem recovery of milk from the same udders after amputation is -0.3579 ± 0.1773 . This coefficient is negative but not significant. Apparently the percentage of post-mortem recovery was not materially influenced by the ante-mortem producing level.

SIGNIFICANCE OF SIZE (EMPTY WEIGHT) OF UDDER

The udders used in this study ranged in weight from 23.30 to 54.15 pounds, and averaged 36.89 pounds. The size, or empty weight of udder, has been shown in a previous paragraph to be negatively or inversely correlated with the characteristics ordinarily associated with udder quality. It also has been shown to be negatively or inversely correlated (-0.6750 ± 0.1107) with percentage of post-mortem recovery of milk from the udders after amputation. Undoubtedly a large udder is essential for liberal production of milk, as it has definitely been shown that most, if not all, of the milk obtained at a milking is present in the udder before milking is commenced. On the other hand, quality of udder is probably of considerable importance, as the smaller udder of good quality seems to be the one that yields its milk most readily on post-mortem milking. Quality may be shown by experiments now under way to indicate more rapid and complete milking in living cows. The question of a relationship between size of udder and capacity of udder should have a practical application and be of general interest. For this group of udders the coefficient of correlation between size and capacity is 0.3444 ± 0.1792 , which is not significant. Apparently a relationship between these two factors does not exist. The coefficient of correlation between size and relation of capacity to weight of udder is -0.5631 ± 0.1389 ,

indicating that in this group the smaller udders have a greater capacity for each unit of weight than do the larger ones.

In a discussion of size or quality of udder, the matter of breed can not be entirely overlooked. The lightest udder was from a Guernsey, the second lightest was from a grade Holstein, and the third and fourth lightest were from Jerseys. The others were from either grade or registered Holsteins. The highest percentage post-mortem recovery of milk was from a grade Holstein, and the lowest was from a Holstein. The highest composite grade for udder quality was assigned to a grade Holstein, the next two highest grades to a Jersey and a grade Holstein, and the two lowest grades to Holsteins. The number of animals of each breed is so small that separate breed correlations could not be obtained. As far as its effect on the results covered up to this point is concerned, however, breed seems to be of little importance.

IMPORTANCE OF UDDER CAPACITY

The method of determining the capacity of the secretory system of cow udders by filling them with formalin and measuring the quantity of fluid they will hold, has been described in this paper. (p. 579.) The capacities of the 11 udders ranged from 28.18 to 65.40 pounds and averaged 50.11 pounds. Since the milk, as it is secreted, appears to be collected and held in the udder until it is withdrawn at milking time, it would appear that the capacity of the secretory system of the udder should be of definite importance.

The fact that most, if not all, of the milk is stored within the udder before milking is commenced, also raises the question of the importance of frequent milking of cows producing heavily. Results in practice indicate the necessity for more frequent milking of high-producing than of low-producing cows. On the other hand, the udder capacities given in Table 1 show that cow udders capable of holding 6 gallons of milk are not uncommon, and indicate ample space for storing in the udder all the milk obtained at a milking. In all probability, however, the udder of the living cow is not capable of holding a quantity of milk as great as the measured capacity of the amputated udder, because at the time of measuring, the blood has been removed from the udder, and because the pressure employed in filling it is probably greater than that of the milk within the living udder. A theory that has been advanced, which seems reasonable and which appears to be supported by scientific data, is that as the milk accumulates, the pressure within the udder gradually increases, and after a certain pressure has been developed, the rate of secretion decreases. In all probability secretion would be entirely stopped before a pressure as great as that used in filling the udder with formalin is reached. If the rate of secretion varies inversely with the accumulation of milk, the most rapid secretion should take place soon after milking and the greatest total secretion should take place in udders that are milked sufficiently often to keep the internal pressure below the point at which maximum activity of the secreting cells begins to be checked. This is in accord with the common practice of milking heavy producers more than twice daily, whereas two milkings daily seem to be sufficient for cows in moderate production.

The udder capacities given in Table 1 for this group of cows range from 28.18 to 65.40 pounds and average 50.11 pounds. Those for

udders 846, 903, and 811 may be a trifle high, as a layer of ice was found beneath the skin when they were sectioned, although not in sufficient quantity to affect materially the capacities given. The udder that was next to the smallest in size (empty weight) but highest in quality, in pounds of milk obtained post-mortem, and in percentage of post-mortem recovery, is very close to the highest in capacity. The three lowest capacities recorded were obtained for the two Jerseys and the Guernsey. Each of the three was below 40 pounds. All the Holsteins, both grade and registered, had udder capacities ranging from 46.36 to 65.40 pounds. It appears that in this study, which, however, includes but few animals, breed is more closely associated with results based on udder capacity than with results based on udder weight or udder quality. For an equivalent butterfat yield, the high percentage butterfat breeds would require less capacity for milk yield than the low percentage butterfat breeds. It seems probable, also, that an udder having a capacity sufficient to hold 50 pounds of 3 per cent milk could accommodate 50 pounds of 6 per cent milk, although the space required for the glands secreting the 6 per cent milk may or may not be greater than that required for the glands secreting an equal quantity of 3 per cent milk.

The capacity of the udder has already been shown to have no relation to the udder-quality grades. The relation of capacity to post-mortem recovery (-0.0516 ± 0.2028) also is of no significance. The correlation between capacity and size of udder (0.3444 ± 0.1792) is extremely doubtful if at all significant. Because of the method by which relation of capacity to weight of udder is determined, a significant and positive correlation between this ratio and udder capacity might be anticipated since capacity has a twofold influence. The coefficient in this case is found to be 0.5591 ± 0.1398 .

SIGNIFICANCE OF RELATION OF CAPACITY TO WEIGHT OF UDDER

As previously stated, relation of capacity to weight of udder was determined by dividing the capacity of the udder by its weight and multiplying the result by 100. In every instance the capacity of the secretory system was greater than the weight of the udder. The percentages showing relation of capacity to weight of udder range from 100.98 to 243.10 and average 142.36. These percentages have been shown to be positively and rather definitely correlated with the udder-quality grades. Their correlation with post-mortem recovery of milk (0.5611 ± 0.1393) and with capacity of udder (0.5591 ± 0.1398) also are positive and significant, but their correlation with size (empty weight) of udder (-0.5631 ± 0.1389) is negatively or inversely significant. The positive correlation of these percentages with capacity of udder, and the similar negative correlation with size of udder, are more or less to be expected on account of the method of obtaining the "relation" percentages, which gives either capacity or weight a twofold influence in determining its correlation with relation of capacity to weight of udder. The positive and relatively significant correlations of relation of capacity to weight of udder with udder quality and with post-mortem recovery of milk indicate a fairly definite association between the three factors.

STAGE OF LACTATION

The stage of lactation of the cows used in this study varied from less than 1 month to more than 23 months when ante-mortem examinations were made, and from 1 month to 24 months when slaughtered. The influence of such a variation in stage of lactation on quality of udder, post-mortem recovery of milk, size (empty weight) of udder, and capacity of udder is logically questioned. It seems probable that the higher quality grades might be associated with advanced lactation, and since high quality and high post-mortem recoveries of milk are so definitely related, that the cows in advanced lactation would be the ones whose udders would milk out most completely post-mortem. Neither of these theories, however, is supported by the analysis of the data. The coefficient of correlation between stage of lactation and the composite grade for udder quality is 0.1280 ± 0.2098 , and that between stage of lactation and percentage post-mortem recovery of milk is -0.0581 ± 0.2027 . The decrease in magnitude of udder with advance in lactation is a common observation which would lead one to anticipate a negative relationship between stage of lactation and size of udder. The coefficient of correlation between these factors is negative but not significant (-0.1578 ± 0.1983). The coefficient of correlation between stage of lactation and udder capacity (0.4295 ± 0.1659) is of doubtful significance. On the whole, these results indicate that the wide variation in stage of lactation within this group of cows was not of great importance as a disturbing factor.

SIGNIFICANCE OF VARIATIONS IN UDDER STRUCTURE

A description of the method of grading the relative size of cistern, the openness of tissue, and the abundance of visible connective tissue, and of measuring the relative size of cistern with the use of a planimeter, has been given. The grades and percentages obtained are listed under "Gross anatomy" in Table 1. A study has been made to determine the relationship existing between each of these gross-anatomy variables and a selected few of the ante-mortem quality grades and post-mortem udder characteristics, the nature of which suggested the possibility of such a relationship.

RELATIVE SIZE OF CISTERN (GRADED)

A positive correlation between the relative size of cistern and some of the udder-quality grades, particularly the free space, has been assumed. It has seemed probable also that size of cistern might be associated with post-mortem recovery, capacity of udder, and relation of capacity to weight of udder but correlated negatively or inversely with size of udder. Coefficients of correlation have been determined to show the degree of relationship between the relative size of cistern (graded) and the following udder characteristics:

Yieldability of udder.....	+0.0355 ± 0.2130
Softness and mellowness of gland tissue.....	+ .5047 ± .1590
Average free space (inches).....	+ .6792 ± .1149
Composite grade for quality.....	+ .4444 ± .1712
Per cent post-mortem recovery.....	+ .3422 ± .1796
Size (empty weight) of udder.....	- .6626 ± .1141
Capacity of udder.....	- .3888 ± .1726
Relation of capacity to weight of udder.....	+ .4224 ± .1671

The relative size of cistern (graded) appears to be distinctly and positively correlated with the average free space; its relation to softness and mellowness of gland tissue is moderate; its relation to composite grade for quality is of very doubtful significance; and its relation to yieldability of udder is of no significance. The relation of relative size of cistern (graded) to size (empty weight) of udder is, as anticipated, significantly negative or inverse, but its relation to percentage post-mortem recovery of milk, capacity of udder, or relation of capacity to weight of udder is of very doubtful significance.

RELATIVE SIZE OF CISTERN (MEASURED)

A high degree of correlation between the grades for relative size of cistern and the corresponding percentages based on planimeter measurements would indicate that the results obtained by the two methods were similar. The coefficient of correlation is 0.8626 ± 0.0521 . On account of the difficulties encountered in measuring the cisterns, and the greater possibility of allowance being made for the smaller divisions in the porous type of cistern when grading is done by visual examination, the grades are probably more nearly accurate than the percentages based on measurements. However, the following correlation coefficients have also been determined to show the degree of relationship between relative size of cistern (measured) and other udder characteristics.

Average free space (inches).....	+0.2554 ± 0.1994
Composite grade for quality.....	+ .2930 ± .1950
Per cent post-mortem recovery.....	+ .3520 ± .1782
Size (empty weight) of udder.....	- .4773 ± .1570
Capacity of udder.....	- .5004 ± .1525
Relation of capacity to weight of udder.....	+ .0316 ± .2032

A low negative or inverse correlation with both size and capacity of udder is suggested, but the others are not significant.

OPENNESS OF TISSUE

It seems reasonable to suppose that openness of tissue would be positively correlated with udder quality, percentage of post-mortem recovery of milk, capacity of udder, and relation of capacity to weight of udder, but negatively or inversely correlated with size of udder. The relationships found to exist are shown by the following coefficients of correlation.

Yieldability of udder.....	-0.1546 ± 0.2082
Softness and mellowness of gland tissue.....	+ .3617 ± .1854
Average free space (inches).....	+ .3604 ± .1856
Composite grade for quality.....	+ .2700 ± .1977
Per cent post-mortem recovery.....	+ .3437 ± .1793
Size (empty weight) of udder.....	- .2004 ± .1952
Capacity of udder.....	- .1131 ± .2008
Relation of capacity to weight of udder.....	- .0364 ± .2031

In every instance in this group, the coefficient is less than twice the probable error. It appears, therefore, that the openness of tissue as graded for this group of udders, is not correlated with udder quality or with any of the other udder characteristics studied.

PROPORTION OF CONNECTIVE TISSUE

"Proportion of connective tissue" refers to the relative abundance of light-colored streaks of tissue which are visible on the cut surfaces of the gross-anatomy sections of the amputated udders. "Abundance of fiber," which has frequently been discussed in connection with udder quality, refers to a condition that is observed at the time of the ante-mortem examination of the udder. It is described in detail at the beginning of this paper. Although the proportion of connective tissue and the abundance of fiber in an udder are judged by entirely different methods, one would anticipate a fairly close relationship between these two factors. Perhaps this so-called connective tissue that is seen in the sectioned udder and the so-called fiber that is felt on examination of the udder are one and the same thing. Plate 11, A, a photograph of a vertical transverse section of the udder of cow 443, is a very good illustration of the appearance of connective tissue in the gross-anatomy sections. It appears here as white streaks resembling somewhat the fatty marbling of beef. The photomicrograph of tissue from the udder of the same cow (pl. 11, B) is a good illustration of the minute structure of connective tissue. It has the appearance of a river, the main stream of which runs from the middle of the picture at the right, in a big bend toward the bottom and back toward the center at the left. In order to determine the relation between the proportion of connective tissue appearing on the cut surface of the gross-anatomy sections and the quality and other characteristics of the udder, the following correlations were derived.

Yieldability of udder.....	+ 0.2884 ± 0.1956
Softness and mellowness of gland tissue.....	- .3126 ± .1925
Abundance of fiber.....	+ .5800 ± .1415
Composite grade for quality.....	+ .2020 ± .2046
Per cent post-mortem recovery.....	+ .1983 ± .1954
Size (empty weight) of udder.....	+ .0432 ± .2030
Capacity of udder.....	- .0980 ± .2014
Relation of capacity to weight of udder.....	- .2394 ± .1917

A fairly significant positive correlation is found to exist between proportion of connective tissue and abundance of fiber. All the other correlations, however, are not significant.

In the entire group of 30 correlations based on grades for gross-anatomy characteristics, only 3 could be considered as definitely significant. Positive correlations are found between size of cistern (graded) and average free space, and between proportion of connective tissue and abundance of fiber. A negative correlation is found between size of cistern (graded) and size of udder. The ability to anticipate accurately the gross structure of the udder by examining it ante-mortem appears from these results to be very limited.

RELATION OF UDDER CHARACTERISTICS TO PRODUCING CAPACITY

The ultimate goal of this general study of the udder is to determine for a large number of cows the relationship of the conformation, quality, gross anatomy, and histology of the udder to its milk and butterfat producing capacity. The data are presented here to complete the information given in connection with the 11 cows used in the post-mortem milking experiments. Data similar to those presented in this paper are being secured on a considerable number of

cows of known producing ability with records made under comparable conditions at the Beltsville experiment station. Unfortunately the production records of these 11 cows were not all made under uniform conditions. The production records were made at ages varying from 2 to 6 years. Moreover, the record of one cow was not complete. In general the milk and butterfat records were made under official testing conditions on three daily milkings, but three of the cows were obtained from another herd where milk production only was recorded and where the cows were kept under average herd conditions and milked twice daily.

TABLE 2.—*Producing capacity of 11 cows included in this study*^a

Cow No.	Breed	Record of production for 1 year								
		Milk	Butter-fat	Age of cow		Times milked daily	Mature equivalent			Numerical rating on production ^b
							Milk	Butter-fat	Milk with additional correction for management	
		Pounds	Pounds	Years	Months		Pounds	Pounds	Pounds	Grade
459	Jersey	10, 153	536	2	1	3	13, 909	712	13, 909	6
292	Holstein	(^c)								
123	Guernsey	8, 535	477	2	5	3	10, 412	581	10, 412	5
272	Holstein	12, 762	447	2	0	3	17, 866	625	17, 866	5
946	Grade Holstein	12, 220		4	9	2	12, 831		19, 246	6
903	do.	12, 943		3	11	2	14, 366		21, 549	7
811	do.	10, 928		4	9	2	11, 474		17, 211	5
257	Holstein	24, 135	852	6	4	3	24, 135	852	24, 135	8
253	do.	16, 405	628	5	8	3	16, 569	634	16, 569	5
443	Jersey	9, 621	465	6	4	3	9, 621	465	9, 621	4
255	Holstein	11, 202	416	2	8	3	11, 202	415	11, 202	3

^a The arrangement of data in this table is the same as in Table 1.

^b As explained in the following pages, the grades in the last column give each cow credit for her production record on the basis of the performance that could be expected of her when breed characteristics are taken into consideration.

^c See comment on record following this table.

^d Record not completed.

In order to place the records as nearly as possible on a comparable basis, adjustment was first made for variations in age by determining the mature equivalent of each production record as made. The age adjustments were made on the basis of factors determined by Fohrman,¹² some of which are as yet unpublished. The milk and butterfat records as made and their mature equivalents are given in Table 2. One point which might otherwise be considered a discrepancy deserves an explanation. The highest production record of cow 459 was made at 2 years 1 month of age. Although she lived to make other records, the mature equivalent is determined on the basis of the first record because she developed a pathological condition in the genital organs which rendered her apparently incapable of subsequent normal reproduction or maximum lactation. The record made at 2 years 1 month of age is, therefore, considered the most accurate index of this cow's inherent producing capacity. On the other hand, cow 255

¹² FOHRMAN, M. H. FACTORS FOR ADJUSTING MILK AND BUTTERFAT RECORDS OF REGISTER OF MERIT JERSEY COWS TO A UNIFORM AGE BASIS. Jour. Dairy Sci. 9: 469-480, illus. 1926.

made several subsequent records and never improved that recorded at 2 years 8 months of age. Since she failed to show any physical condition which might explain her failure to improve the first-lactation performance, it is used also as her mature equivalent without adjustment. Woodward¹³ has shown that cows kept under official-test conditions and milked and fed three times daily produce approximately 50 per cent more milk and butterfat than do the same cows when kept under average herd conditions. The milk-production records of cows 846, 903, and 811, which were made on two milkings daily and under more or less average herd conditions, were again adjusted to allow for conditions of management by the addition of 50 per cent to their mature equivalents. The adjusted records for these three cows together with the mature equivalent milk records for the others are presented in a separate column in Table 2. As a basis for studying the relation of the characteristics of the udder to the quantity of milk produced by it, these data are the best obtainable.

Coefficients of correlation have been determined to show the relation between each of the 14 udder characteristics under consideration and the milk production of the cow expressed in pounds, as finally adjusted for age and conditions of management. These correlation coefficients, with the udder characteristics designated, follow:

Looseness of udder.....	+ 0.0200 ± 0.2247
Yieldability of udder.....	- .3484 ± .1975
Softness and mellowness of gland tissue.....	- .2076 ± .2151
Abundance of fiber.....	- .3704 ± .1940
Average free space (inches).....	+ .0229 ± .2247
Composite grade for quality.....	- .1665 ± .2186
Per cent post-mortem recovery.....	- .2168 ± .2033
Size (empty weight) of udder.....	+ .5467 ± .1496
Capacity of udder.....	+ .6379 ± .1265
Relation of capacity to weight of udder.....	+ .1406 ± .2091
Relative size of cistern (graded).....	- .4017 ± .1789
Relative size of cistern (measured).....	- .2311 ± .2019
Openness of tissue.....	- .0526 ± .2127
Proportion of connective tissue.....	- .4071 ± .1779

In this group of 14 items the coefficient is less than its probable error in 6 cases, less than twice the probable error in 4 other cases, and less than three times the probable error in 2 other cases. None of the quality grades shows any significant correlation to production of milk. In only two instances, capacity of udder and size of udder, is the correlation significant, and the latter is only moderately so. These correlations of both capacity of udder and size of udder to milk-producing ability indicate that the high producer possesses an udder of greater size and capacity than does the low producer. This does not necessarily mean that the size or capacity of the udder is proportional to the total yearly production, as a large total yearly production record may result from a high maximum with a relatively rapid decline, or from a more moderate maximum and greater persistency. The correlations shown, however, indicate that the udder must be of sufficient size and capacity to hold the amount of milk secreted between milkings. This is in harmony with previous observations of the writers¹⁴ that most, if not all, of the milk obtained at a milking is held in the udder before the milking process is commenced.

¹³ WOODWARD, T. E. INFLUENCE OF TWO PLANES OF FEEDING AND CARE UPON MILK PRODUCTION. *Jour. Dairy Sci.* 10: 283-291, 1927.

¹⁴ SWETT, W. W., MILLER, F. W., and GRAVES, R. R. *Op. cit.*

The foregoing consideration of the relation of udder characteristics to producing capacity was based entirely on quantity of milk produced and made no allowance whatever for butterfat production or for breed characteristics. A quantity of milk that would represent high production for a Jersey or Guernsey would be considered close to average for a Holstein, and the two Jerseys and the one Guernsey were all located in the lowest four positions in the alignment on which the previous consideration was based. Undoubtedly some of these cows occupy positions considerably below what they would have if butterfat production and breed characteristics had been considered.

It is desirable that a similar study should be made of the relation of quality grades and other characteristics of the udder to producing capacity, in which each cow is given credit for production on the basis of the performance that could be expected of her if breed characteristics were taken into consideration. Such credits could be based with reasonable fairness on butterfat production if the actual butterfat production were known in all cases. For three of the cows the percentage of butterfat was not determined when the milk records were made. Although the approximate richness of their milk was known, and their butterfat production could be approximated, in view of the lack of definite records of butterfat production it was thought best to use the grading system instead of basing the correlation coefficients on the butterfat yield. The results would probably be essentially the same in either case.

The 9-point grading system described in this paper was employed. With primary attention directed to the known and estimated butterfat production, but with the milk production records and the breed characteristics of each cow in mind, the cows were placed in one of six production groups ranging from very high to low, and each cow was assigned an equivalent numerical grade. The grades assigned appear in the last column of Table 2. The six groups used and their equivalent numerical grades are as follows: Very high production, 8; high production, 7; medium to high production, 6; medium production, 5; medium to low production, 4; and low production, 3.

Coefficients of correlation have been determined to show the degree of relationship between the 14 udder characteristics under consideration and the numerical ratings assigned to represent the production performance of each cow. Udder characteristics are listed below in the order in which they appear in Table 1, and each is followed by the coefficient of correlation indicating the extent of relationship with the performance grades.

Looseness of udder.....	+ 0.1381 ± 0.2205
Yieldability of udder.....	- .4166 ± .1858
Softness and mellowness of gland tissue.....	- .1765 ± .2178
Abundance of fiber.....	- .4969 ± .1693
Average free space (inches).....	- .0703 ± .2237
Composite grade for quality.....	- .2174 ± .2142
Per cent post-mortem recovery.....	+ .0039 ± .2133
Size (empty weight) of udder.....	+ .2348 ± .2015
Capacity of udder.....	+ .2059 ± .2043
Relation of capacity to weight of udder.....	+ .0337 ± .2131
Relative size of cistern (graded).....	- .2279 ± .2022
Relative size of cistern (measured).....	+ .0620 ± .2125
Openness of tissue.....	+ .0878 ± .2116
Proportion of connective tissue.....	- .4426 ± .1715

Apparently not one of the characteristics of the udders studied is distinctly indicative of the cow's ability or lack of ability to perform, as shown by the performance ratings. In 7 of the 14 items the coefficient is less than its probable error; in 4 other cases it is less than twice its probable error; and in the 3 remaining items it is less than three times its probable error, although 1 is very close to the ratio of 3 to 1. The coefficient for abundance of fiber is of only moderate significance and might be indicative of the undesirability of abundant fiber in the udder. It is worthy of note also that the next highest coefficient is for proportion of connective tissue. The abundance of fiber was judged in the living cow. The proportion of connective tissue was judged from the photographs of the gross-anatomy sections. The fact that these two correlations are both negative and that they are the highest in the entire group might be interpreted as indicating that, as has been assumed, the fiber of the living udder and the visible connective tissue in the gross sections are either closely related or are the same, and also that they are not beneficial and may be detrimental to high production.

Both size of udder and capacity of udder were found to be fairly definitely correlated with the cow's ability to produce milk, but were not correlated with the performance grades which give consideration to butterfat production. This fact suggests again the comment made under the discussion of udder capacity, that for an equivalent butterfat yield the breeds having a high butterfat test would require less capacity for milk yield than the breeds having a low butterfat test.

DISCUSSION OF THE UDDER CHARACTERISTICS, LIFE HISTORY, AND PERFORMANCE OF EACH COW

COW 459 (PRODUCTION GRADED AS MEDIUM TO HIGH)

Cow 459 was a registered Jersey. Her record of 10,153 pounds of milk and 536 pounds of butterfat was made under official testing conditions at the age of 2 years 1 month. She failed to equal this record again, but on account of the fact that she developed a pathological condition which rendered her apparently incapable of subsequent normal reproduction or maximum lactation, the mature equivalent of this 2-year-old record, which amounts to 13,909 pounds of milk and 712 pounds of butterfat, was used as a measure of her producing capacity. She was slaughtered in the third month of lactation at 8 years of age. Her udder was very high in composite grade for quality, relatively low in size (empty weight), had the smallest capacity in the group, a very low relation of capacity to weight of udder, and yielded a high percentage of ante-mortem production on post-mortem milking.

Gross anatomy.—This udder shows a very large, open, and rather definitely circumscribed cistern. The gland tissue is very open and porous, and the relatively large ducts are distributed over nearly all the surface of the sections. An average quantity of connective tissue is visible.

Histology.—Sections from a front quarter of the udder showed many of the alveoli to be in a state of functional inactivity in this portion of the gland. There was an abnormal amount of fibrous tissue in this quarter and many of the lobules showed deposits of numerous small concretions, or milk calculi, in the alveoli. The histological

appearance of the rear quarter indicated normal functional activity of this portion of the gland. In certain areas there was some loosening and sloughing of the epithelial lining of the acini, and a few small inflammatory centers were noted. The area illustrated in Plate 2 was taken from the central part of the left rear quarter, which was functioning but which was noticeably smaller and narrower than the other quarters.

General.—This cow was a persistent aborter. At the last five parturitions one calf was born that died at 2 days of age and the other four were abortions of single or twin fetuses. She bred normally, but apparently was unable to retain a fetus after it reached a certain size. This probably accounts for the fact that her maximum production was made at 2 years of age, for the record indicated is sufficient to show beyond doubt that she had an inheritance for good production. Available records fail to show any udder disturbance requiring treatment. The possible influence of abortion on either gross anatomy or histology of the udder is entirely speculative. The significance of age on structure also is speculative. The large cistern and open structure of gland tissue may have been at least partly responsible for the relatively high post-mortem recovery of milk.

COW 292 (PRODUCTION NOT GRADED)

Cow 292, a registered Holstein, did not have a complete yearly record of production, but she was lactating at an average 2-year-old level when slaughtered in the second month of lactation at 2 years 3 months of age. This cow was killed for this work, because she did not fit into the breeding projects and space was needed. Her udder was relatively very low in composite grade for quality, relatively high in size (empty weight), less than average in capacity, low in relation of capacity to weight of udder, and very low in percentage post-mortem recovery of milk.

Gross anatomy.—This udder has an average sized and distinctly circumscribed cistern. The gland tissue is open in the lower part, relatively dense above and of nearly average porosity as a whole. The visible connective tissue is very limited in quantity.

Histology.—The appearance of this gland, for the most part, indicated normal functional activity. One section from the central area of a rear quarter showed some evidence of chronic inflammatory changes, as indicated by a perceptible increase in the interstitial tissue in the areas involved. The fibrous framework of the gland in sections from normal areas was unusually soft or loose in structure. The area illustrated in Plate 3 was taken from a point near the base and about midway between the front and rear quarters on the left side.

General.—Examination of the living udder indicated that it was normal, except that the right rear quarter was slightly larger than the others. There is no record of this cow's having had udder disturbances requiring treatment. An udder at this age would hardly be expected to show excessive connective tissue, and should be actively functioning at this stage of lactation. Relationships between producing ability and udder characteristics are not obtainable, owing to the early age at which the cow was slaughtered.

COW 123 (PRODUCTION GRADED AS MEDIUM)

Cow 123, a registered Guernsey, produced 8,535 pounds of milk and 477 pounds of butterfat under official testing conditions at the age of 2 years 5 months. The maturity equivalent based on this production is 10,412 pounds of milk and 581 pounds of butterfat. She was slaughtered in the seventh month of lactation at the age of 4 years 3 months. Her udder was about medium in composite grade for quality, the lowest in the entire group in size (empty weight), next to lowest in capacity, about average in relation of capacity to weight of udder, and almost exactly average in percentage post-mortem recovery of milk.

Gross anatomy.—This udder has a very large and unusually even and distinctly circumscribed cistern above which the gland tissue is of about average density and somewhat less than average in proportion of visible connective tissue. After treatment with formalin and sectioning, the tissue was flexible, soft, watery and almost mushy.

Histology.—In sections from a front quarter of the udder the alveoli were seen to be well developed, indicating normal functional activity. Some evidence of degeneration of the glandular epithelium was noted in several of the sections. All the sections from the rear quarter showed an inactive state of the glandular tissue. The various lobules showed a marked increase in the interlobular connective tissue of the quarter. On account of the entirely different conditions existing in the right rear as compared with the other quarters (see next paragraph), the histological structure of both the right front (functioning) and the right rear (inactive) quarter is illustrated in Plate 4.

General.—The right rear quarter was indurated and entirely inactive and was described as relatively small and consisting only of a fibrous cylinder extending toward the teat. Undoubtedly this accounts for the histological notations for that quarter. Apparently the other quarters were functioning normally. There is no other record of this cow's having had udder disturbances requiring treatment.

COW 272 (PRODUCTION GRADED AS MEDIUM)

Cow 272, a registered Holstein, made a production record of 12,762 pounds of milk and 447 pounds of butterfat at 2 years of age on three milkings daily. This is equivalent to a mature production of 17,866 pounds of milk and 625 pounds of butterfat. She was in the seventh month of lactation and was 4 years 10 months of age at the time of slaughter. She was destroyed at this early age because she had been pronounced permanently sterile. Observations on the living udder are not available for this cow. The amputated udder was one of the largest in size (empty weight) of those examined, was almost average in capacity, very low in relation of capacity to weight of udder, and yielded the lowest percentage of ante-mortem production on post-mortem milking.

Gross anatomy.—In size of cistern this udder ranks considerably below the average. The teat canal and cistern are continuous and well circumscribed. The tissue is very dense and large ducts are confined to a small area above the cistern. An average quantity of fairly well distributed connective tissue is visible. The ducts were white and shiny, and the gland tissue after formalin treatment and sectioning was soft, wet, flabby, and sloppy.

Histology.—Histological examination of this udder showed a mixture of functioning and nonfunctioning lobules of the gland in the different areas sectioned. There was a limited increase in the fibrous connective tissue of the organ. A number of milk concretions were seen in different lobules. The histological area illustrated in Plate 5 was taken from a point near the base of the left front quarter.

General.—Available records failed to indicate any udder disturbance requiring treatment during a previous lactation, but the left front quarter was described as small. During the preliminary 10-day milking period immediately before slaughter, however, a hardened and congested condition developed in the left front quarter which was accompanied by a greatly reduced flow of milk from that quarter. After two days of treatment by massaging, the condition of the udder and the quantity of milk appeared to be about normal but the milk was ropy. After three days of treatment the udder and the milk appeared to be entirely normal.

COW 846 (PRODUCTION GRADED AS MEDIUM TO HIGH)

Cow 846, a grade Holstein, produced 12,220 pounds of milk at 4 years 9 months of age on two milkings daily. The mature equivalent of this record is 12,831 pounds, and an additional adjustment for management raises it to 19,246 pounds. This cow was slaughtered in the fifth month of lactation at 6 years 2 months of age. Her udder was very good to excellent in quality, ranking first in the group on the basis of composite grade. It was next to the smallest in size (empty weight), close to the highest in capacity, and by far the highest in relation of capacity to weight of udder. It also yielded by far the highest proportion of ante-mortem production on post-mortem milking.

Gross anatomy.—The cistern is large and definite in outline with many large ducts leading from it. The tissue is above the average in openness and porosity and has very little visible connective tissue. The gland tissue after treatment with formalin and sectioning was soft and flexible and very wet.

Histology.—Sections from this udder showed a rather heavy fibrous framework, the interstitial connective tissue being soft or loose in texture. A few of the lobules in both front and rear quarters showed a state of inactivity, but for the most part this gland appeared to have been functioning normally. The area illustrated in Plate 6 was taken from a point near the base of the left front quarter.

General.—The history of udder disturbances for this cow is lacking, but the ante-mortem examination failed to show any abnormality or lack of uniformity in condition indicative of such disturbances.

COW 903 (PRODUCTION GRADED AS HIGH)

Cow 903, a grade Holstein, produced 12,943 pounds of milk at about 3 years 11 months of age on two milkings daily. The mature equivalent of this production is 14,366 pounds for the conditions under which it was made, or 21,549 pounds when adjusted for conditions of management. This cow was slaughtered in the fifteenth month of lactation at 5 years 2 months of age. Her udder was rated very high in composite grade for quality, very close to average in size (empty weight), the highest of the group in capacity, near the

top in relation of capacity to weight of udder, and remarkably close to average in percentage post-mortem recovery of milk.

Gross anatomy.—The cistern consists of a honeycomblike mass of very small and more or less independent cavities, is indefinite in outline and appears to be small in total volume. This udder is above the average in openness of structure of gland tissue and shows more than the average quantity of connective tissue. After treatment with formalin and sectioning, the gland tissue was soft, flexible, very wet, and sloppy. For several days after the sections were cut a milky white exudate continued to flow from the cut surface of the tissue.

Histology.—The fibrous framework of this udder was found to be very soft or loose in texture. There was considerable degeneration of the epithelial tissue, and in many of the lobules there was sloughing of the epithelial lining of the alveoli. In places one or more of the alveoli had been completely destroyed and in some areas they had apparently been replaced by fat tissue. A section from the superior portion of the rear quarter showed groups of inactive lobules. Groups of alveoli partly or completely filled with pus cells were seen in a number of the sections. The area illustrated in Plate 7 was taken from a point near the base of the left front quarter.

General.—The history of udder disturbances for this cow is lacking, but ante-mortem examination failed to show evidence of such disturbance, abnormality, or lack of uniformity in condition except that the udder was "relatively light" in the left rear quarter.

COW 811 (PRODUCTION GRADED AS MEDIUM)

Cow 811, a grade Holstein, produced 10,928 pounds of milk on two milkings daily at about 4 years 9 months of age. The mature equivalent of this production is 11,474 pounds for the conditions under which it was made, or 17,211 pounds if adjusted for conditions of management. She was slaughtered about 24 months after her last calving, at the age of 6 years 9 months. Her udder was only slightly below the average in composite grade for quality, slightly below the average in size (empty weight), next to the highest in the group both in capacity and in relation of capacity to weight of udder, and considerably below the average in percentage of ante-mortem production recovered on post-mortem milking.

Gross anatomy.—The cistern is about average in size, somewhat divided, and indistinct in outline. The gland tissue appears to be of average openness of structure and to have only a small quantity of connective tissue. The udder tissue was generally very flexible, soft, and watery when sectioned.

Histology.—This udder showed evidence of infection. Many of the alveoli and a number of the milk ducts were filled with pus cells. There was some degeneration and sloughing of the alveolar epithelium, and the sections from the various areas showed an increase in the interlobular connective tissue. Many of the alveoli were filled with concretions, pus cells, and cell detritus. Because of the abnormal conditions in the left front quarter (see next paragraph), histological sections from both front and rear quarters are illustrated in Plate 8.

General.—The previous history of udder disturbances for this cow is lacking, but when the cow was obtained for this study the left

rear quarter of her udder was noticeably small and stringy, and produced less milk than the other quarters, which were relatively full and round. A few days before she was slaughtered garget developed in the left front quarter, and the milk from that quarter diminished in quantity and became thick and ropy. In all probability this accounts for the presence of pus cells and to some extent explains the other indications of degeneration noted in connection with the histological examination of the udder.

COW 257 (PRODUCTION GRADED AS VERY HIGH)

Cow 257, a registered Holstein, produced 24,135 pounds of milk and 852 pounds of butterfat under official-testing conditions at the age of 6 years 4 months. A previous record made under similar conditions at 3 years 1 month of age amounted to 20,357 pounds of milk and 702 pounds of butterfat. She was slaughtered in the second month of lactation at the age of 10 years 9 months. Her udder was very low in composite grade for quality, the largest in size (empty weight) in the group, considerably above the average in capacity, the lowest in relation of capacity to weight, and considerably below the average in percentage of ante-mortem production recovered on post-mortem milking.

Gross anatomy.—A few ducts of medium size are present, but the quarter shown is almost devoid of a definitely formed cistern. The volume of the cistern or the few ducts above the teat is extremely small. The structure of the gland tissue is of about average openness and the amount of visible connective tissue rather small. The tissue after treatment was spongy, rubbery, and fairly typical. The structure was unusually uniform in all the sections of this udder.

Histology.—This udder showed chronic inflammatory changes, as indicated by extensive round cell infiltration and a noticeable increase in the interalveolar and interlobular connective tissue. The increase in the interstitial tissue resulted in atrophic changes or decrease in the number and size of many of the alveoli. Many of the acini showed evidence of inactivity. In this connection the advanced age and heavy production of this cow should be taken into consideration. The area illustrated in Plate 9 was selected from a point near the base of the right rear quarter of the udder.

General.—An examination of records failed to show any disturbance during the life of the cow requiring treatment of the udder. The udder appeared to be normal and was functioning in all quarters. The ante-mortem examination indicated the presence of fiber which was relatively abundant and harsh, especially in the rear quarters, and no evidence of open cisterns. These observations are supported by the histological findings which indicate abundant connective tissue, and by the fact that the gross-anatomy sections showed an extremely small cistern.

COW 253 (PRODUCTION GRADED AS MEDIUM)

Cow 253, a registered Holstein, produced 16,405 pounds of milk and 628 pounds of butterfat under official testing conditions at the age of 5 years 8 months. The mature equivalent of this record would be 16,569 pounds of milk and 634 pounds of butterfat. She was slaughtered 14 months after her last calving, at the age of 9 years.

4 months. Her udder was about average in composite grade for quality, close to the highest in size (empty weight), above the average in capacity, considerably below the average in relation of capacity to weight, and slightly above the average in percentage of post-mortem recovery of milk.

Gross anatomy.—The cistern is below the average in relative size, and is divided and indistinct in outline. The tissue is dense and there is a very large quantity of connective tissue. The appearance of the udder may have been affected slightly by the fact that the udder was not well frozen when sectioned and as a result was somewhat torn by the saw. Udder 257, however, was in almost the same condition as regards completeness of freezing and showed a small quantity of connective tissue. After formalin treatment and sectioning the tissue was very wet, inclined to be sloppy, and lacking in firmness and spongy consistency.

Histology.—Sections from this udder showed only a limited amount of normal functioning glandular tissue. There had been some chronic irritation of the gland which resulted in a general increase in the connective tissue of the organ. The entire organ showed very marked degenerative and atrophic changes. Many of the alveoli showed detachment of the epithelium, and the lumen of a number were filled with the denuded epithelial cells and cellular débris. A great many milk calculi were observed. In places practically one-half of the acini were filled with these concretions. The area illustrated in Plate 10, was selected from a point near the center of the left rear quarter of the udder.

General.—When the udder was examined in February, 1926, the right front teat was distended with pus, and in March of the same year the same teat and quarter were described as hard. When the udder was examined before slaughter, the left rear quarter was slightly larger and hung lower than the others, but the udder was functioning in all quarters. The very abundant fiber noted on ante-mortem examination is in accord with the histological and gross-anatomy findings. The free space grading is also generally supported by the rating for size of cistern.

COW 443 (PRODUCTION GRADED AS MEDIUM TO LOW)

Cow 443, a registered Jersey, produced 9,621 pounds of milk and 465 pounds of butterfat under official testing conditions at the age of 6 years 4 months. Apparently this is indicative of her maximum producing ability. Her udder was about average in composite grade for quality, small in size (empty weight), low in capacity, close to average in relation of capacity to weight of udder, and next to the highest in the group in post-mortem recovery of milk.

Gross anatomy.—The cistern is proportionately very small and of the honeycomb rather than the distinctly circumscribed type. The gland tissue is very dense, free of visible ducts, and has unusually abundant connective tissue throughout. This udder, like Nos. 257 and 253, was not completely frozen when sectioned. The tissue after sectioning was very soft, wet, and lacking in firmness and in the usual spongy and rubbery consistency.

Histology.—In this udder sections from all the different areas in the front and rear quarters showed a number of abnormal changes.

The connective tissue framework of the gland had a soft, spongy appearance suggestive of an edematous condition of the organ. All the sections showed more or less detachment of the epithelial lining of the acini, and chronic inflammatory changes were also noted. In places whole groups of lobules had apparently been replaced by fat tissue. Many concretions varying in size were seen in the alveoli in the different sections. The area illustrated in Plate 11 was selected from a point near the base of the left front quarter.

General.—As far as records show, this udder had never experienced any noticeable disturbance. When examined before the cow was slaughtered, it was classed as in good condition. There were indications of large cisterns in all except the right rear quarter, which is the quarter used in illustrating the gross structure. (Pl. 11, A.) The histological findings can not be explained by any ante-mortem notations. The relatively advanced age of this cow should be considered in this connection, and the use of petroleum ether in the half subsequently used for histological study is noted.

COW 255 (PRODUCTION GRADED AS LOW)

Cow 255, a registered Holstein, produced 11,202 pounds of milk and 415 pounds of butterfat under official testing conditions at 2 years 8 months of age. In a later official test at 7 years 3 months she produced 11,480 pounds of milk and 392 pounds of butterfat. As her mature record did not exceed the record made at 2 years 8 months, the mature equivalent was not calculated. This cow undoubtedly represents the lowest producing capacity of any cow in the group. She was slaughtered one month after last freshening at the age of 9 years 7 months. Her udder was very slightly above the average in composite grade for quality, above the average in size (empty weight), well above the average in capacity, slightly below the average in relation of capacity to weight of udder, and about average in percentage of post-mortem recovery of milk.

Gross anatomy.—The cistern is of about medium size, very much divided into relatively large chambers, and indistinct in outline. The tissue is very open in structure and shows a large quantity of connective tissue. The tissue after treatment was very wet and inclined to be soft and mushy.

Histology.—This udder showed only one outstanding deviation from the normal, consisting of an infiltration of white blood cells into and between the alveoli of the gland. These cellular infiltrations, which were composed largely of eosinophile cells, were observed in all the sections examined. The fibrous framework of this gland was of very soft or loose texture. The area illustrated in Plate 12 was obtained from a point near the top of the left front quarter.

General.—The udder apparently was sound. It tilted forward slightly and the left side was somewhat heavier than the right. Records fail to show any disturbance requiring treatment of the udder at any time.

SUMMARY AND CONCLUSIONS

Separate grades for quality, based on ante-mortem examination of the udder, have been assigned to looseness of udder, yieldability of udder, softness and mellowness of gland tissue, abundance of fiber,

and average free space. A composite grade for all of these items is used in some parts of this study as a single indication of udder quality.

The size (empty weight) of the udders used in this study varied from 23.30 to 54.15 pounds and averaged 36.89 pounds.

The table of capacities shows that a cow's udder after amputation may be capable of holding as much as 6 gallons of milk. Six of the 11 udders had capacities of more than 50 pounds, and the average for the entire lot was 50.11 pounds. This indicates ample space for storing in the udder all the milk obtained at a milking. There is some probability, however, that the pressure resulting from the accumulation of milk in the udder gradually checks and finally stops milk secretion. If this is true, the most rapid secretion should take place soon after the udder is emptied, and the greatest total production should be obtained from cows milked often enough to keep the internal udder pressure sufficiently low to enable milk secretion to continue unchecked. This may explain why heavy producers yield greater quantities when milked three or four times than when milked only twice daily.

The relation of capacity to weight of udder is an index of the udder's fluid-holding capacity for each unit of empty weight, and should indicate its porosity. The percentages showing this relationship range from 100.98 to 243.10 and average 142.36. In other words, the capacity was greater than the weight in every instance, and on an average was nearly one and one-half times the weight of the empty udder.

It appears that a definite and positive correlation exists between udder quality and post-mortem recovery of milk after amputation of the udder. This is not only true for the composite grade for quality, but for all except one of the individual quality items, and indicates that milk can be drawn more readily from an amputated udder of good quality than from one of poor quality. Experiments are now in progress which should indicate whether or not high quality of udder is conducive to rapid milking in the living cow.

Udder quality and size (empty weight) of udder seem to be more or less negatively or inversely correlated. Every one of the correlations determined was negative. Those for yieldability of udder and composite grade for quality were significant, those for softness and mellowness of gland tissue and average free space were moderate in degree, and those for looseness of udder and abundance of fiber were not significant. On the other hand, a moderate positive correlation was shown to exist between size of udder and total yield of milk.

Apparently udder quality is not in any way associated with udder capacity so far as this group of udders is concerned.

Udder quality appears to be positively and rather definitely associated with relation of capacity to weight of udder. Every one of the correlations was positive. Those for yieldability of udder, average free space, and composite grade for quality were significant, that for softness and mellowness of gland tissue was very nearly significant, and those for looseness of udder and abundance of fiber were not significant.

A significant and negative or inverse correlation was found to exist between post-mortem recovery of milk and size (empty weight) of udder. This indicates that the milk was drawn more readily from the small udders than from the large ones. The small udder of good

quality seems to be the kind that yields its milk most readily on post-mortem milking.

Post-mortem recovery did not appear to be in any way associated with capacity of udder.

The post-mortem recovery was fairly definitely associated with relation of capacity to weight of udder. This suggests again a probable positive relationship between quality of udder and relation of capacity to weight of udder.

Apparently the post-mortem recovery of milk from amputated udders was not materially affected by the ante-mortem level of production at the time of slaughter.

Size (empty weight) of udder and capacity of udder do not appear to be definitely correlated.

The small udders had a greater capacity for each unit of empty weight than did the large ones.

Breed characteristics appear to have had little effect on the results shown in connection with udder quality, post-mortem recovery of milk, or size (empty weight) of udder but to have had some effect on the results based on udder capacity.

Stage of lactation in this group of cows does not seem to be associated with composite grade for quality, percentage of post-mortem recovery of milk, size (empty weight) of udder or capacity of udder. Apparently, therefore, the variation in stage of lactation was not of great importance as a disturbing factor.

Relative size of cistern (graded) appears to be positively and fairly definitely correlated with free space; its correlation with size (empty weight) of udder was distinctly negative or inverse; and its correlation with other udder characteristics was either moderate or not significant. The relative size of cistern (measured), which was particularly difficult to determine, was only moderately correlated with any of the udder characteristics studied in relation to it.

The relative openness or porosity of the gland tissue was not correlated with udder quality or with any of the other udder characteristics studied.

The relative abundance of connective tissue visible in the gross anatomy sections of the amputated udder, was positively and fairly definitely correlated with abundance of fiber which was graded ante-mortem, but does not appear to be correlated with any of the other udder characteristics studied.

The possibility of anticipating the gross structure of the udder by examining it ante-mortem appears from these results to be very limited.

The producing ability of the cows used in these studies as indicated by their milk records after adjustment for age and management, seems to be moderately correlated with size (empty weight) of udder, and distinctly correlated with udder capacity. The correlations between milk-producing ability and other ante-mortem and post-mortem udder characteristics were all very doubtful or of no significance. This consideration was based entirely on quantity of milk produced and made no allowance whatever for butterfat production or for breed characteristics.

Numerical ratings designed to give credit for the cow's efficiency as a producer by making allowance for the influence of breed charac-

teristics, were assigned to the production performance of each cow. These ratings were to a very large extent based on butterfat production. Correlation coefficients, determined to show the degree of relationship between these numerical grades and 14 different udder characteristics, were either of very doubtful significance or of no significance whatever. Little relationship was found to exist between ratings for production and udder characteristics.

The histological findings can in most cases be accounted for when consideration is given to the history of the udder, either through its stages of activity or its observed condition at and immediately before slaughter.

In most of these cases the period that elapsed between the completion of the production record given and the slaughtering of the cow, was long enough to provide ample time for the development of the abnormal conditions of the udder tissue reported in some of the histological examinations. It would be interesting to know how much the producing capacity of udders would be impaired by such abnormal developments in the mammary tissue. It seems desirable, therefore, to make histological examinations of the udder tissue of some high-producing cows soon after the completion of their production records.

The limitation in number of animals available for the study presented in this paper is fully appreciated. The results presented are more or less preliminary and are not conclusive. Additional studies of many of these considerations based on larger populations are under way.

RELATION OF THE SEMIPERMEABLE MEMBRANES OF THE WHEAT KERNEL TO INFECTION BY GIBBERELLA SAUBINETII¹

By GRACE WINELAND PUGH, formerly *Assistant Pathologist*, and HELEN JOHANN, *Associate Pathologist, Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*, and JAMES G. DICKSON, *Professor of Plant Pathology, University of Wisconsin*, and Agent, *Division of Cereal Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture*²

INTRODUCTION

Relatively little has been published on the formation of the so-called semipermeable membranes of the caryopsis of the Gramineae and their relation to kernel development and maturation. Likewise, the penetration of the cellulose and ligno-subero-cellulose cell walls and cuticularized epidermal surfaces of the kernel and the relation of these structures to invasion of the tissues by various microorganisms have not been studied in detail. Do such membranes in the cereal kernel play an important rôle in preventing aggressive invasion of the embryo and storage structures in the later stages of kernel infection? Are these membranes partly accountable for the presence of *Fusarium* and other organisms of this type in the outer structures of the kernel, as is so frequently found to be the case in lightly infected grain? Since not only the extent of the infection, but its location as well, largely determines the quality of the infected grain, it seemed especially important to study the kernel development in relation to the formation of these structures and their rôle in limiting infection by the wheat-scab parasite.

The results reported in this paper were obtained in connection with a general study of cereal *Fusaria*. The infected kernels studied were from naturally infected and artificially inoculated heads, fixed in 1923 and 1924 and worked up by the senior writer at that time. The infections were obtained on immature and mature kernels, the stage of development being determined by observation checked by later histological examination. The infected mature kernels were secured after a period of rainy weather from bundles of cut grain lying on moist ground where the fungus, either from natural infections or from artificial inoculations, had overrun the heads after the grain was ripe. Inoculations of mature wheat heads standing in the field or in the greenhouse made in connection with this study were unsuccessful, owing in all probability to the fact that no technic had then been perfected for supplying adequate moisture throughout the incubation period of the fungus. It has seemed advisable, however, to summarize the investigations and present the results in this paper.

¹ Received for publication Dec. 18, 1931; issued November, 1932. Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the Wisconsin Agricultural Experiment Station.

² The writers wish to express their thanks to Eugene H. Herrling, Department of Plant Pathology, University of Wisconsin, who made a large proportion of the photomicrographs used.

The difficulties usually experienced in cutting serial sections of mature kernels of the cereals were overcome by treating the killed and partially dehydrated grains with a mixture of equal parts of chemically pure hydrofluoric acid and 70 per cent alcohol for from 48 to 72 hours. Thorough washing in water for 24 hours, followed by the usual steps in the paraffin method of embedding, prepared the material for sectioning. Delafield's haematoxylin and erythrosin following Juel's zinc chloride fixative³ gave a color contrast between host and fungus. Acidified Sudan III⁴ was used to demonstrate the presence of suberized or cutinized parts.

HISTORICAL REVIEW

General interest in these semipermeable layers of the kernel was awakened by Brown (2)⁵ in 1907 in his work with barley. He used *Hordeum vulgare* var. *coerulescens*, which has a blue pigment in the aleurone cells that is turned red by acid, thus furnishing a natural indicator for the study of permeability in relation to acids. In his conclusions he states (1) that the grain of *Hordeum* is inclosed within a semipermeable or selective covering; (2) that the semipermeable property of the covering of the grain of *Hordeum* is not due to the action of living protoplasm; (3) that the semipermeable property of the covering of the grain of *Hordeum* is located in the spermoderm of the grain; and (4) that the grain of *Avena*, *Triticum*, and *Secale* is inclosed in a semipermeable covering apparently similar to that of the grain of *Hordeum*.

The exact location of the semipermeable property of the covering was not known for some time. In publishing on later researches, Brown (3, p. 84), in 1909, says:

Histological study of the seed-coverings indicates that their selective power is confined to the testa, and probably to that portion which is derived from the epidermis of the nucellus during the development of the seed.

Schroeder (8), working with wheat in 1911, was of the opinion that penetration by liquids was not uniform over the surface of the grain. He thought that entrance was effected chiefly at the embryo end or that there was increasing impermeability of the coverings from the basal toward the apical end of the grain. Eckerson (5), working with wheat in 1917, spoke of the outer membrane of the nucellus as a semipermeable membrane.

Collins (4), working with barley in 1918, was the first to study seriously the structure of the integumentary system in relation to localized water absorption and semipermeability. He steeped the grains in water for two days and then cut transverse sections through the mid region by hand. Concerning structure in the region of the furrow he says (4, p. 384-385):

The furrow corresponds in position and extent with an elongated chalazal tract, through which nutriment and reserve materials pass from the vascular supply in the ovary wall to the cells of the endosperm. The tissues of the pericarp and ovule are continuous; indeed this elongated tract is to be regarded as the base of the ovule—the extended chalaza from the flanks of which the integu-

³ Juel's fixative: Zinc chloride, 10 g; acetic acid, 10 g; 50 per cent alcohol, 500 c c.

⁴ Acidified Sudan III: Saturated solution of Sudan III in 70 per cent alcohol; 8 to 10 drops of H₂SO₄ per 100 c c of solution.

⁵ Reference is made by number (italic) to Literature Cited, p. 625.

ments originate. On the ovular side of the vascular bundle, and lying immediately between the points of origin of the tegmen, is a group of cells of glandular character with somewhat thickened walls and yellowish homogeneous contents. In the developing grain the contents appear to be of an oily nature. Radiating from this group, towards the centre of the developing grain, is a sheaf like mass of elongated cells which serve to distribute supplies to the endosperm.

The epidermis of the nucellus extends, he says, immediately within the tegmen, up to its origin, but not across the chalazal tract itself. As the nucellar epidermis does not delimit the cells of sheaflike structure, it can not obstruct the passage of salts at this point.

Concerning the extent of the outer membrane of the tegmen, his sections of immature ovules showed that it completely enveloped the ovule except at the micropyle and in the chalazal tract. In longitudinal sections of the mature grain the micropylar point was not satisfactorily made out, although the membranes could be traced up to a dark-colored caplike covering to which was attached the embryonic appendage. Concerning the embryonic appendage he says (4, p. 385):

This is a group of cells forming the apex of the root-sheath, which, lying immediately beneath the micropyle, makes real contact between the embryo and the tegmen. The cells differ from those of the root-sheath proper in that they have no contents and are capable of very rapid swelling when placed in contact with water. Although the actual origin of these cells has not been proved yet, the author is of the opinion that they may represent a suspensorial group.

He says that real contact between tegmen and membrane is also made "over the peripheral rim of the scutellum through the agency of a single layer of modified aleurone cells." He concludes in relation to the entrance of water—

that the micropyle is the point of rapid entry and the seat of differential action through the agency of the embryonic appendage, whilst slow, differential filtration takes place across the chalazal tissue lying along the furrow,

movement of liquids being most rapid at the point "where it meets the dorsal margin of the scutellum."

Braun (1), in 1924, found a gradient of permeability to iodine in the wheat seed coats. He says the entrance of iodine takes place over the entire surface of the grain, radially, but not uniformly. The apparent lateral and distal spread from the embryo end is due to the existence of a gradient of permeability to iodine in the seed coats, which permeability is greatest near the embryo end, diminishes to a minimum near the distal end, and again increases slightly at the distal apex. Microtome sections of immature wheat seeds fixed in Flemming's strong solution showed a deep blackening of the cutin layer near the distal end with scarcely any discoloration at the embryo end and a gradient of discoloration between. In mature seeds the cutinization gradient was found to be nearly the same as that determined by the use of Flemming's triple stain.

The chemical nature of the semipermeable membrane or membranes has been variously described. Eckerson (5) says the semipermeable membrane of wheat is suberized. Collins (4) found that the tegmen membranes of barley grains were cuticularized at an early stage of development. Gordon (6), in her study of the development of the endosperm in cereals, calls the testa of barley a layer impregnated with silica, and Braun (1) speaks of a cutin layer in connection with his studies of permeability to iodine in the wheat seed coats.

A report of a study on the anatomy of wheat kernels infected with a *Fusarium*, made in 1889 by Woronin, is found in a publication by Palchevsky (7) which appeared in 1891. Woronin carried on investigations with wheat infected with a *Fusarium* which he called *F. roseum* Link and which he considered to be the conidial stage of *Gibberella saubinetii*. He notes that sometimes infection is localized only in the embryo and on adjacent parts while the rest of the kernel remains normal. Concerning the anatomy of the kernel infected before maturity he states:

The anatomical picture of the wheat kernel is quite different from that of the normal grain. The mycelium, which is often of an intense purple color, penetrates from the periphery of the kernel through the seed coat and the aleurone layer into the endosperm of the seed, spreading in all directions. The shrunken periphery of the kernel, seen through a microscope, is wavy, and the epidermis of the ovary sometimes remains green (chlorophyll), for the fungus attacks kernels that are not ripe.

The pigment layer of the kernel, slightly darker than normal, forms accumulations here and there. Between the pigment and the aleurone layer, that is, in the place of the layer of thick-walled cells, there is a dense mycelium which often surpasses the seed coat in thickness and occasionally forms tumors.

The aleurone layer is wavy, interrupted, and has the appearance of a dark stripe; there are many dark spots in its contents, and in general it is darker than normal; however, it stains normal crimson with SO_3 (A. S. purum) with sugar sirup. In such grains containing mycelium the cell structure, especially of the seed-coat layers, is destroyed. The starch grains are connected by fungus threads, like clusters of grapes; the smaller grains seem to be firmly attached to them.⁶

STRUCTURE OF KERNELS

MORPHOLOGICAL ORIGIN OF THE TESTA

The testa is derived from the inner integument of the ovule. In the very young grain, both the inner and the outer integuments consist of two layers of cells. (Fig. 1, A.) The outer integument disintegrates and disappears very early in the development of the grain. The inner integument changes more slowly (fig. 1, B) and persists in a modified form. Before the developing kernel occupies the entire nucellar space, sections stained with Sudan III show very thin red lines, membranes of equal thickness on both borders of the inner integument. (Fig. 2, A.) The membrane on the outer wall thickens rapidly (fig. 2, C); the inner one remains so thin that in mature kernels it is likely to be overlooked. These membranes are unaffected by the ordinary cytological stains. Their reaction to Sudan III and their solubility in sulphuric acid indicate the presence of suberinlike or cutinlike compounds in the walls and on the outer surface.

As development proceeds, the outer cells of the integument lose most of their contents and collapse. At the same time the contents of the inner row collect in masses which seem to contain globules at first and which later have a homogeneous waxy or oily appearance. (Fig. 1, B; fig. 2, C.) The masses take on color as they collect, varying from golden to golden brown. The walls of these cells also become brownish. As the kernel matures the remains of the two rows of cells of the inner integument are crushed into a continuous narrow golden-brown layer commonly called the color layer of the wheat

⁶ Translation by I. V. Krasovskiy



FIGURE 1.—A, Cross section of a portion of a wheat flower: *a*, Anther; *o*, ovary; *d*, outer integument; *i*, inner integument. Stained with Flemming's triple stain. $\times 130$. B, Cross section showing the respective layers of an immature wheat kernel: *ep*, outer epidermis of pericarp; *p*, pericarp; *c*, chlorophyll (cross) layer; *s*, outer membrane of the testa; *i*, cells of inner integument; *n*, epidermis of nucellus; *al*, aleurone cells forming; *en*, endosperm. Stained with Delafield's haematoxylin and erythrosin. $\times 300$

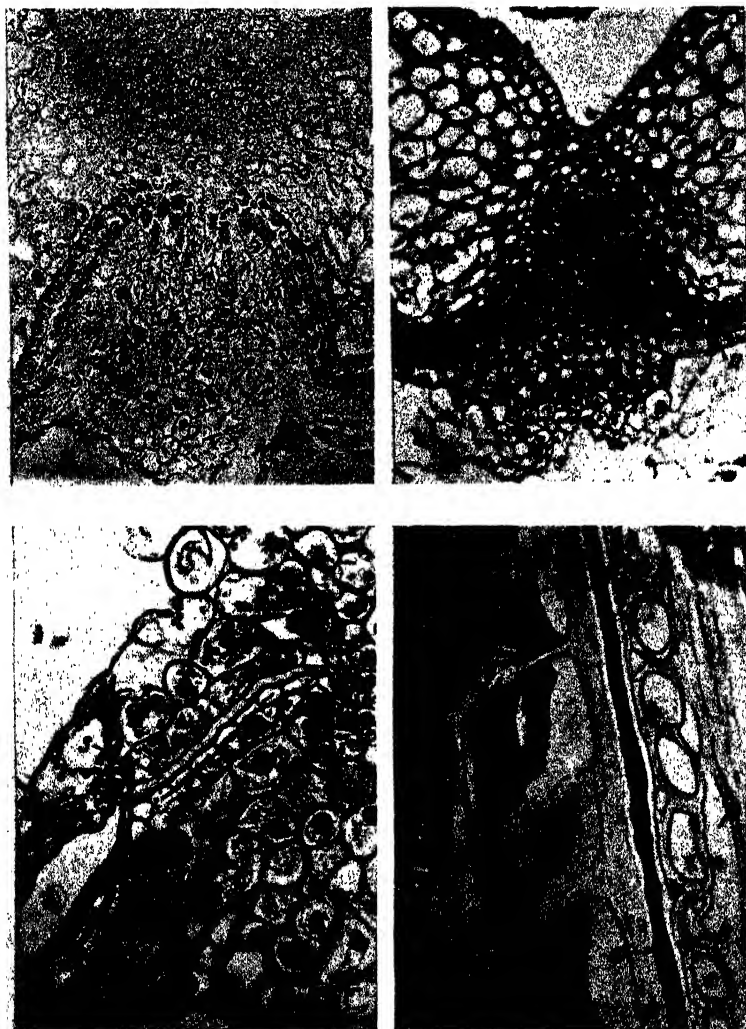


FIGURE 2.—A, Cross section through the groove region of a young kernel, showing the points of origin of the testa, the two rows of cells of the integument (*i*) bordered by membranes of equal thickness, *s* and *s'*; *n*, epidermis of nucellus; *v*, vascular trace of groove; *tr*, transchalazal area. Stained with Sudan III. $\times 178$. B, Cross section of the transchalazal region in the groove of an immature kernel, showing the location of the points of origin of the inner integument and the epidermis of the nucellus; *p*, pericarp; *i*, inner integument; *n*, epidermis of nucellus. Stained with Delafield's haematoxylin and erythrosin. $\times 104$. C, Cross section of a portion of an immature kernel, showing the thick membrane along the outer wall of the outer row of cells of the inner integument near its point of origin in the groove; *s*, outer membrane of testa; *i*, cells of inner integument; *n*, epidermis of nucellus; *p*, pericarp. Stained with Flemming's triple stain. $\times 328$. D, Longitudinal section showing width and structure of the several layers in a mature wheat kernel; *s*, outer membrane of testa; *i*, color layer of testa; *s'*, inner membrane of testa; *n*, epidermis of nucellus; *al*, aleurone cells; *en*, endosperm; *c*, chlorophyll cells, cross layer, of pericarp; *p*, parenchyma of pericarp. Stained with Delafield's haematoxylin and erythrosin. $\times 535$

grain. (Fig. 2, D.) The compression process in the filling of the kernel is completed when the grain is mature and is completed last at the basal end of the seed and in the groove on either side of the chalazal region. This layer together with the membranes bordering it on either side constitutes the testa of the mature kernel.

EXTENT OF THE TESTA

As noted previously, the testa of the mature grain is derived from the inner integument of the ovule (fig. 2, B) and is coextensive with it. Both cross (fig. 3, C) and longitudinal (fig. 3, B) sections of kernels show that it envelops the seed except at the micropyle and in the extended chalazal region of the groove (fig. 3, A). Cross sections of a kernel midway between the embryo and the brush end show that it originates sharply at either side of the vascular trace of the groove. (Fig. 4, A.)

Along the groove between the lines of origin of the testa are cells which have some of the characters of the cells of the inner integument, but whose exact nature was not determined. Collins speaks of them in barley as "cells of glandular character." The contents of these cells appear to pass through the changes described for the contents of the cells of the inner integument in the formation of the color layer, becoming oily or waxy in appearance and golden brown in color. The changes first appear in scattered cells, beginning shortly after the corresponding changes in the inner integument. They seem to progress less rapidly and are completed last at the base of the chalazal region. (Fig. 5, A and C.) When the kernels are mature, the transchalazal area is filled with a compact group of cells similar to the color layer in the appearance of the cell contents. The walls of these cells are thicker than the adjacent parenchyma of the pericarp, and stain a deep red with safranine (fig. 5, A), as do the cell walls in the color layer (fig. 2, C). When treated with acidified Sudan III, they stain a light red not observed in the compressed color layer, indicating the presence of suberin or cutin. These membranes, like the layers of the testa, are quite resistant to the action of 72 per cent H_2SO_4 .

Cells whose contents appear to be similar to those of the transchalazal area are found bordering the micropyle, but here they are clearly a part of the color layer. A cross section through the micropyle indicates that the color layer folds outward and back at the opening and is connected at its end with the end of the outer layer of the testa. (Fig. 5, D.) In the vicinity of the micropyle the testa does not undergo compression as is the case in the regions bordering the endosperm, and hence the color layer in this region does not lose its appearance of cellular structure. (Fig. 5, D; fig. 4, C.) Collins states that the walls bounding the micropyle were found to be cuticularized. It was not clear from the sections examined whether the thin inner membrane follows the surface of the color layer as it folds outward at the micropyle, but probably it does.

RELATIVE THICKNESS OF THE OUTER LAYER OF THE TESTA

The outer layer of the testa of the wheat kernel is not of uniform thickness throughout its extent. (Fig. 6, A and C.) The thickest portions are found in the groove, along the top of the kernel where it

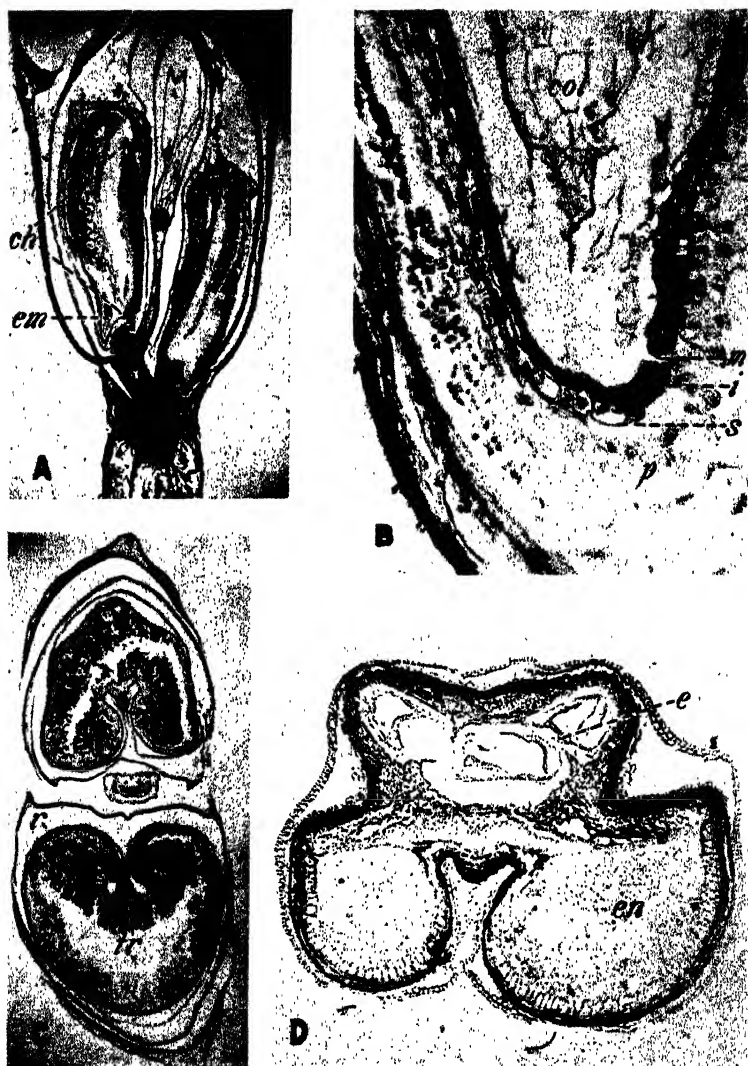


FIGURE 3.—A, Longitudinal section of a spikelet containing two kernels, showing the location and extent of the chalazal region (*ch*) and the position of the embryo (*em*). Stained with Flemming's triple stain. $\times 9$. B, Longitudinal section through the radicle of an immature kernel, showing the opening through the testa at the micropyle; *s*, outer membrane; *i*, cells of inner integument; *m*, opening of the micropyle; *col*, coleorhiza; *p*, pericarp. Stained with Flemming's triple stain. $\times 220$. C, Cross section of a spikelet containing two kernels, showing the location of the testa (*t*), transchalazal group of cells (*tr*), and the vascular trace of the groove (*v*). Stained with Flemming's triple stain. $\times 8$. D, Cross sections of an infected kernel, showing the resulting destruction of the embryo; *e*, embryo; *en*, endosperm. Stained with Delafield's haematoxylin and erythrosin. $\times 22$



FIGURE 4.—A, Cross section through the groove showing the points of origin of the testa (*s* and *i*), also the scattered cells in the transchalazal region (*u*) which have taken on a golden-brown color similar to that of the color layer (*c*); *r*, vascular trace of the groove. X 135. B, Portion of a longitudinal section through the embryo of a wheat kernel, showing the relatively thick outer membrane (*o*) of the testa at the base of the chalazal region (*ca*); *s*, inner membrane of testa. The cells of the inner integument (*i*) do not undergo severe compression at this point. Stained with Sudan III. X 205. C, Longitudinal section through the micropyle (*m*) of a mature wheat kernel; *s*, outer membrane of testa; *i*, color layer (inner integument). Stained with Sudan III. X 375.

passes under the style (fig. 5, B), and in the region extending from the micropyle to the base of the groove (fig. 4, B). It is thinnest over the embryo. Serial sections show that its thickness is fairly uniform in the groove from the basal to the brush end of the kernel.

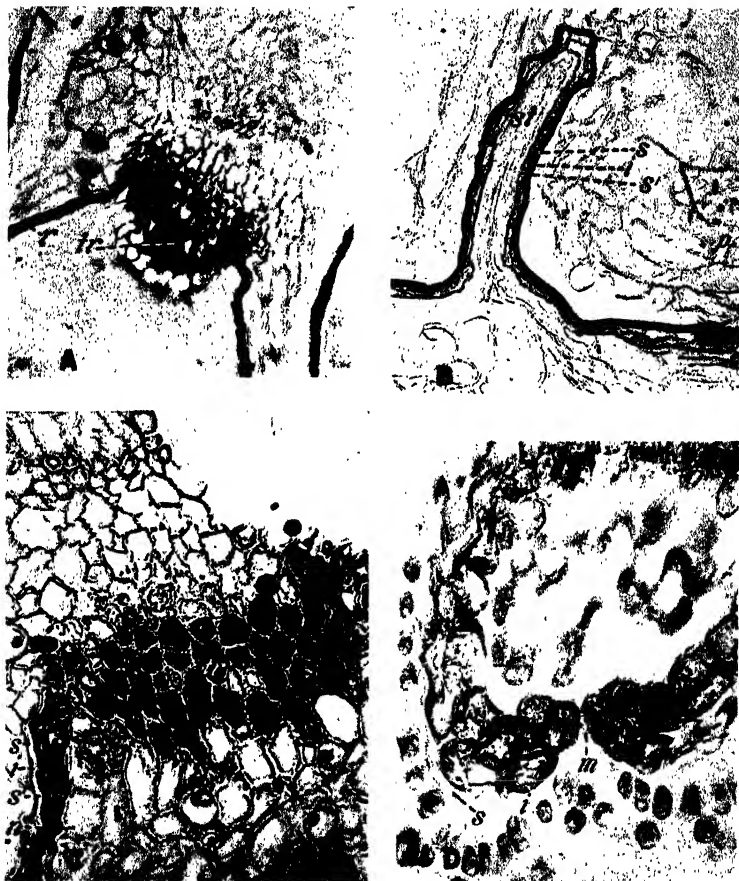


FIGURE 5.—A, Cross section through the groove, above the embryo, of a fairly mature wheat kernel, showing the testa (*t*) and the tranchalazal group of cells (*tr*); the walls of both color layer and tranchalazal cells and much of the cell contents stain red with safranin; *v*, vascular trace of the groove. Stained with Flemming's triple stain. $\times 75$. B, Portion of a longitudinal section of a kernel, showing the thickness of the outer membrane of the testa under the remains of the style (*st*); *s*, outer membrane; *i*, color layer of testa; *s'*, inner membrane of testa; *p*, pericarp. Stained with Sudan III. $\times 173$. C, Cross section through a portion of the chalazal region at the embryo end of the kernel, showing the slightly thickened walls and the deeply stained cell contents of the tranchalazal group (*tr*); *s*, outer membrane of testa; *i*, color layer; *s'*, inner membrane of testa; *n*, epidermis of nucellus; *v*, vascular trace of groove. Stained with Flemming's triple stain. The walls of the tranchalazal cells in this region do not stain as deeply with safranin as do those midway of the same kernel, shown in A. $\times 317$. D, Cross section through the opening of the micropyle (*m*) of a mature wheat kernel; *i*, color layer; *s*, outer membrane of testa. Stained with Delafield's haematoxylin and crythrosin. $\times 288$

On the dorsal side, where the membrane is thin, there is a gradual but slight increase in thickness up to the brush end, where a more abrupt increase is found.

EXTENT OF THE EPIDERMIS OF THE NUCELLUS

In immature kernels the epidermis of the nucellus is seen as a row of cells lying immediately beneath the inner integument of the ovule and may be traced to its lines of origin along the groove. (Fig. 2, A,

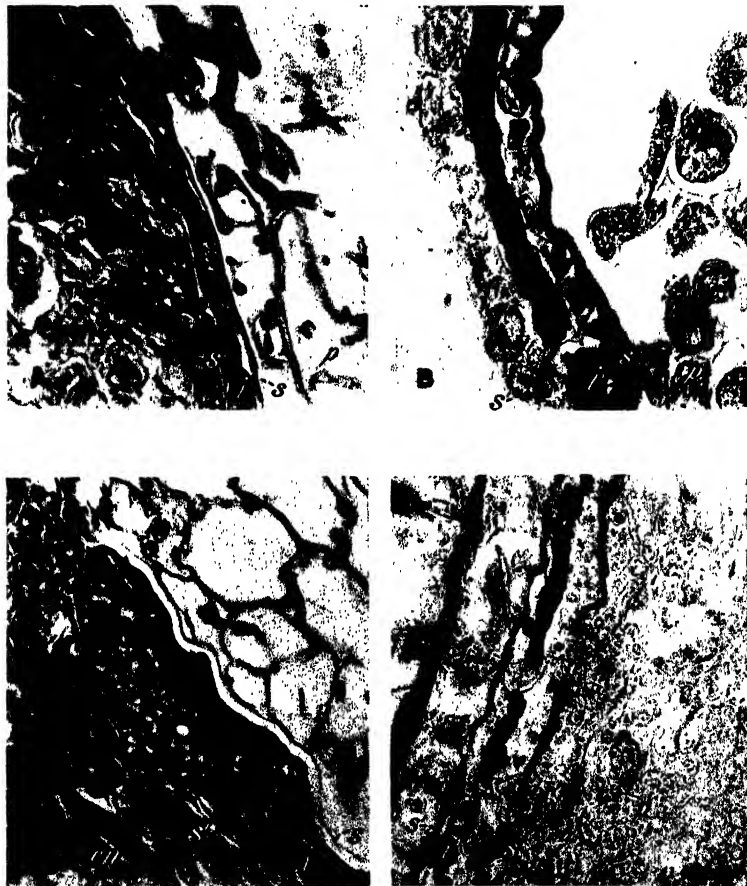


FIGURE 6.—A and C, Cross sections showing the relative thickness of the outer membrane of the testa over the embryo of the mature wheat kernel (A) and in the groove region (C) of the same section of an infected kernel. In C hyphae are so massed in the testa that the two membranes *s* and *s'* are widely separated; *i*, color layer; *en*, endosperm; *p*, pericarp. Stained with Delafield's haematoxylin and erythrosin. $\times 385$. B, Portion of a longitudinal section through the immature and uninfected second kernel of a spikelet. $\times 467$. D, Section through the same region in the infected basal kernel of the spikelet. Comparison of the thickness of the outer membrane of the testa in the two kernels indicates that infection of the basal kernel occurred at a considerably earlier stage of development than that pictured in kernel B. Stained with Sudan III and gentian violet. $\times 350$

B, and C.) In mature kernels it is compressed and appears as a band without visible cellular structure. (Fig. 2, D.) According to Collins (4), the epidermis of the nucellus in barley does not cross the chalazal region. The same is true of wheat. However, in some sections of mature kernels a band similar in appearance but irregular in

width may be found bridging the groove at some distance from the ends of the testa and connecting with the epidermis of the nucellus on either side. Apparently this irregular band is the compressed remains of nucellar tissue which did not disintegrate during the process of maturing.

RESISTANCE TO INFECTION

In correlating the tissues of the wheat kernel with infection it may be said, in general, that the testa becomes increasingly resistant to penetration by *Gibberella saubinetii* as the grain matures. In cases of early infection the membranes may be penetrated in numerous places, as shown in Figure 6, D. Figure 6, B, pictures a location similar to that of Figure 6, D, in the uninfected younger second kernel of the same spikelet. The comparison of Figure 6, B and D, indicates that infection of kernel B occurred at an early stage in its development. The cellular structure of the color layer in B shows the second kernel to be immature, and the greater thickness of the outer membranes of the testa indicates that infection in the older basal kernel, D, must have occurred at a considerably earlier stage of development than that now shown in kernel B.

The outer layer of the testa, the semipermeable membrane, is the part of the kernel most resistant to penetration. It may often be followed in parts of kernels so badly infected that other tissues can scarcely be identified except by their position (fig. 7, C), and it is not unusual to find it intact for considerable distances with mycelium in contact with one or both surfaces (fig. 7, A). Numerous sections show the fungus permeating the color layer and at times becoming so thickly massed there as to force the two membranes of the testa widely apart. (Fig. 6, C.) In such cases it seems probable that the hyphae had entered this layer before it became compressed into a narrow continuous band. In the early stages of development there is no visible barrier to entrance into this layer at its point of origin in the groove region. (Fig. 2, A and B.)

After the outer membrane of the testa has become thickened sufficiently to offer some resistance to the entrance of the fungus there is a period in which the young wheat kernel seems to be relatively unprotected in the groove region. (Fig. 2, A.) As development proceeds, the group of cells in the chalazal region takes on color (fig. 4, A) and the walls thicken slightly and fill the gap between the points of origin of the testa. It does not appear that in the immature kernel this area offers as effective resistance to hyphal penetration as does the outer layer of the testa; nevertheless it serves as a barrier of marked efficiency during the later stages of development of the kernel. (Fig. 5, A.)

The degree of resistance of the membranes of the testa is seemingly proportional to their thickness. Hence, penetration of the outer membrane occurs more often in the region over the embryo than elsewhere. (Fig. 6, A and D.) Though the outer membrane of the testa is the most resistant part within the kernel, it is not impenetrable in any location, and in cases of severe infection hyphae may pass through even the comparatively thick portions. (Fig. 7, B.)

The compressed nucellus shows little more invasion than the testa, owing probably not so much to resistance as to the fact that its thick-

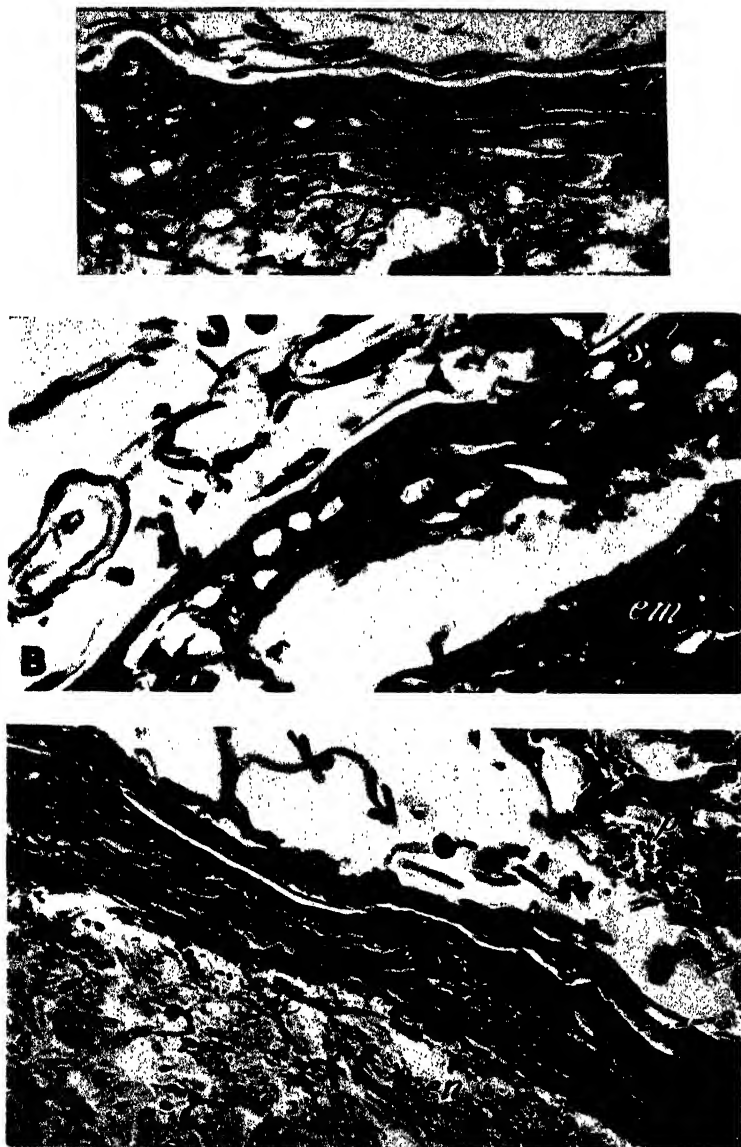


FIGURE 7.—A, Portion of a cross section in the groove of an infected kernel. The outer membrane of the testa (*s*) is intact for considerable distances, though fungus hyphae (*f*) are in contact with both surfaces. Stained with Delafield's haematoxylin and erythrosin. $\times 446$. B, Portion of a longitudinal section of a wheat kernel containing a badly infected embryo; this section is at the flank margin of the embryo, where the outer membrane of the testa is comparatively thick; hyphae have penetrated the membrane in numerous places; *em*, embryo; *i*, color layer of testa; *s*, outer membrane of testa; *p*, pericarp. Stained with Delafield's haematoxylin and erythrosin. $\times 670$. C, Portion of a cross section of a badly infected immature wheat kernel; the outer membrane of the testa (*s*) is visible for considerable distances; the color layer, inner membrane of the testa, epidermis of the nucellus, and aleurone cells are indistinguishable in the mass of hyphae; *en*, endosperm; *p*, pericarp. Stained with Delafield's haematoxylin and erythrosin. $\times 446$

ness and chemical composition make it less attractive to the fungus and hence somewhat of a barrier to hyphal advance.

The scarcity of fungus in the endosperm probably is explained by the lack of sufficient moisture to make growth possible and to the utilization by the fungus of pentosan materials more readily than hexose compounds rather than to any resistance in that area.

DISTRIBUTION OF THE FUNGUS WITHIN THE KERNELS

The fungus penetration of the wheat kernel at the brush end appears to be accomplished with little difficulty at flowering time, when hyphae advance from infected anthers to the ovary. The fungus may permeate the tissue of the kernel quite thoroughly, for the hyphae travel rapidly along the intercellular spaces, and the thin-walled cells of the parenchyma offer little resistance to penetration. At that immature stage the membranes of the testa are thin and the transchalazal group of cells shows little specialization, which leaves the young seed poorly protected. Under such conditions it is usual to find the fungus advancing from the pericarp into the young kernel. The result is that when infections occur at flowering time a comparatively high percentage of kernels are so badly diseased as to be practically worthless.

The distribution of the fungus in kernels infected after they have reached maturity is generally as follows: The pericarp with a light infection throughout or at least at the embryo end; the testa comparatively clean, with the possible exception of penetrations in the embryo region where the outer membrane is thin; the epidermis of the nucellus comparatively clean though showing more infection than the testa; hyphae in some cases massed between the various layers, separating them more or less widely (fig. 8, A and B); the aleurone cells filled with fungus (fig. 8, A) in the embryo region and for a considerable distance toward the brush end in the groove, a shorter distance on the dorsal side, and a much shorter distance on the flanks; the endosperm slightly infected near the embryo; and the embryo almost completely destroyed by the fungus (fig. 3, D.)

It is significant that late infections are more or less localized in the embryo end of the grain, where the outer layer of the testa remains thin and the color layer has not undergone severe compression, and where there is also the opening at the micropyle. Furthermore, it seems possible that the entrance of mycelium at the base of the chalazal region (fig. 3, A; fig. 4, B) might result in the prior infection of the embryo region. One such case was observed. In most cases, however, the embryos were so badly infected that it was impossible to discover the point at which the fungus had first entered the tissues.

Cases were observed in which, at various points along the groove, mycelium was apparently making its way from the pericarp through the irregularly placed cells which stain like the color layer. Perhaps this explains the common infection of the aleurone cells toward the brush end in the groove rather than at other points.



FIGURE 8.--A, Section of an infected wheat kernel, showing hyphae (*f*) massed in the aleurone cells (*al*) on either side of the nucellar layer (*n*) and penetrating the latter in a few places; *en*, endosperm; *p*, pericarp; *s*, outer membrane of testa; *i*, color layer of testa. Stained with Delafield's haematoxylin and erythrosin. $\times 600$. B, Portion of a longitudinal section of a wheat kernel, showing fungus massed between the testa and epidermis of the nucellus, separating them widely in places; *n*, epidermis of nucellus; *a'*, inner membrane of testa; *i*, color layer of testa; *s*, outer membrane of testa; *al*, aleurone cells; *f*, hyphae; *p*, pericarp. Stained with Delafield's haematoxylin and erythrosin. $\times 940$

DISCUSSION

The distribution of fungus in the wheat kernels infected after maturity is somewhat like the advance of "blueing" described by Collins (4, p. 400). He used iodine in potassium iodide and two varieties of barley. He states:

In both cases the first signs of blueing of the starch contents appeared on each side of the furrow at the germ or basal end. From these points it was easy to trace the path taken by the iodine. Whilst it spread to some extent along each side of the furrow toward the apex of the grain, it passed more rapidly around the starchy endosperm rim immediately beneath the scutellum. The two blue arcs joined around the embryo, completing the circuit; at the same time the blueing spread rapidly on the curved side of the grain towards the apex, the lateral edge of colour as a rule being somewhat sharply delimited. The flanks of the furrow were the last to be blued.

The sections used in the present study showed numerous instances in which the forming color layer of the testa was permeated by hyphae. Concerning the passage of solutes through this layer Collins says (4, p. 411-412):

Careful consideration has been given to the possible passage of solutions along the cells of the tegmen, between its inner and outer cuticularized membranes, and the selective action of the inner. The inner cuticle is less prominent and resistant, and might fail after a time. The embryonic appendage might serve as a temporary hindrance to the passage of solutes into the embryo, whilst the main stream would pass between the cuticularized membranes and be distributed in the distal direction. It should be said here that such a method of distribution assumes that the connecting anticlinal walls between the inner and outer membranes at the micropyle fail to resist the passage of solutes. In the ovule these walls bounding the micropyle were found to be cuticularized.

In speaking of the mature kernel he says (4, p. 413):

The initial uptake of water supplies the need of the embryo. * * * The subsequent distribution of liquid in the endosperm is precisely the path of enzyme disintegration within the endosperm during germination of the embryo.

From the evidence at hand it seems probable that several factors influence the distribution of fungus hyphae within the kernel, i. e., the relative resistance of different parts to fungal penetration, the location of readily available food, and the distribution of water within the kernel.

In the very young wheat kernel, hyphae may be found in all parts. At this stage the membranes and cells are not sufficiently developed to offer any great resistance to the advance of the fungus; likewise, available food and moisture are plentiful. *Gibberella* has the ability to utilize a variety of food substances, especially pentosans; hence, the distribution of the abundant hexose nutrients within the kernel probably is only a minor factor influencing the location of the fungus. On the other hand, the location and structure of the protective parts, especially the layers of the testa, and the distribution of moisture within the grain appear to be important factors influencing the entrance and spread of *Gibberella sarbinetii* within the wheat kernels.

SUMMARY

The testa of the mature wheat kernel is derived from the inner integument of the ovule and is coextensive with it. At maturity it consists of a narrow, homogeneous, golden-brown, oily-appearing layer formed by the compression of the two rows of degenerating cells of the inner integument and bordered on the inner side by a very thin color-

less membrane and on the outer side by one of considerable thickness. Both membranes stain with Sudan III and are relatively resistant to sulphuric acid, indicating the presence of suberinlike or cutinlike substances.

The testa completely envelops the seed except at the micropyle and in the groove region, where it is seen to originate sharply at either side of the vascular trace of the groove. In the chalazal region between the lines of origin of the testa there is a group of cells which have some of the characters of the cells of the inner integument but whose exact nature was not determined.

The outer layer of the testa varies in thickness. The thickest portions are found in the groove, along the top of the kernel where it passes under the style, and in the region extending from the micropyle to the base of the groove. It is thinnest over the embryo. On the dorsal side there is a gradual but slight increase in thickness up to the brush end.

In mature kernels the epidermis of the nucellus is compressed into a band without visible cellular structure. It does not cross the groove of the kernel.

The testa becomes increasingly resistant to penetration by *Gibberella saubinetii* as the grain matures. The degree of resistance of the membranes seemingly is proportional to their thickness. The outer membrane of the testa is the most resistant layer of the kernel.

Kernels infected at flowering time may be permeated throughout by the fungus.

In wheat grains infected at maturity the fungus is usually localized at the embryo end of the kernel; it is sparse in the testa, nucellar layer, and endosperm; the aleurone cells may be filled with hyphae for considerable distances along the groove, for a shorter distance on the dorsal side, and a considerably shorter distance on the flanks; the embryo is more or less completely permeated by fungus hyphae.

The location and structure of the protective parts, especially the layers of the testa, and the distribution of water within the kernel appear to be important factors influencing the entrance and spread of *Gibberella saubinetii* within the wheat kernel.

LITERATURE CITED

- (1) BRAUN, H.
1924. A GRADIENT OF PERMEABILITY TO IODIN IN WHEAT SEED COATS. (A PRELIMINARY NOTE). Jour. Agr. Research 28:225-226.
- (2) BROWN, A. J.
1907. ON THE EXISTENCE OF A SEMI-PERMEABLE MEMBRANE ENCLOSING THE SEEDS OF SOME OF THE GRAMINEAE. Ann. Bot. [London] 21:[79]-87.
- (3) ————
1909. THE SELECTIVE PERMEABILITY OF THE COVERINGS OF THE SEEDS OF HORDEUM VULGARE. Roy. Soc. [London], Proc., Ser. B 81:82-93, illus.
- (4) COLLINS, E. J.
1918. THE STRUCTURE OF THE INTEGUMENTARY SYSTEM OF THE BARLEY GRAIN IN RELATION TO LOCALIZED WATER ABSORPTION AND SEMI-PERMEABILITY. Ann. Bot. [London] 32:[381]-414, illus.
- (5) ECKERSON, S. H.
1917. MICROCHEMICAL STUDIES IN THE PROGRESSIVE DEVELOPMENT OF THE WHEAT PLANT. Wash. Agr. Expt. Sta. Bul. 139, 20 p., illus.

-
- (6) GORDON, M.
1922. THE DEVELOPMENT OF ENDOSPERM IN CEREALS. Roy. Soc. Victoria,
Proc. 34 (n. s.):[105]-116, illus.
- (7) PALCHEVSKY, N. A.
1891. [DISEASES OF CEREAL CROPS OF SOUTH URSURIA]. 43 p., illus.
St. Petersburg. (Part II. Results of investigations carried out by
M. S. Woronin. p. 12-19.)
- (8) SCHROEDER, H.
1911. ÜBER DIE SELEKTIV PERMEABLE HÜLLE DES WEIZENKORNES. Flora
[Jena] (n. F. 11) 102:[186]-208, illus.

A THREE-YEAR STUDY OF THE CHEMICAL COMPOSITION OF GRASS FROM PLOTS FERTILIZED AND GRAZED INTENSIVELY¹

By J. G. ARCHIBALD, *Assistant Research Professor of Chemistry*, and P. R. NELSON² and E. BENNETT,³ *Research Assistants, Massachusetts Agricultural Experiment Station*⁴

INTRODUCTION

This paper is based on results obtained in 1928, 1929, and 1930 from an extensive trial of the so-called Hohenheim system of pasture management on the Massachusetts State College farm. Two papers⁵ have been published giving the results for 1928 and 1929, respectively. This paper combines the results for all three seasons. The variations in the schedule of fertilizer application in the different years are shown in detail in Table 1.

TABLE 1.—*Schedule of fertilizer applications used each year, 1928, 1929, and 1930*

Year, plot group, and treatment	Elements applied	Pounds per acre applied	Dates of application
1928			
A, Completely fertilized; grazed throughout the season.	Nitrogen (N).....	85	Apr. 20-May 5.
	Phosphoric acid (P ₂ O ₅).....	55	
	Potash (K ₂ O).....	67	
	Nitrogen (N).....	10	At intervals throughout the season, varying from 30 to 58 days; commencing May 28, ending Sept. 6.
do.....	10		
B, completely fertilized but cut for hay in June; grazed from July 1.	Nitrogen (N).....	85	May 7 and 8.
	Phosphoric acid (P ₂ O ₅).....	55	
	Potash (K ₂ O).....	67	
	Nitrogen (N).....	10	At intervals throughout the season, varying from 20 to 66 days; commencing June 21, ending Sept. 15.
do.....	10		
do.....	10		
D, control plot.....	No fertilizer.....		
1929			
A and B.....	Nitrogen (N).....	41	Apr. 20-May 2.
	Phosphoric acid (P ₂ O ₅).....	41	
	Potash (K ₂ O).....	50	
	Nitrogen (N).....	15	At intervals throughout the season, varying from 26 to 56 days; commencing May 28, ending Aug. 29.
do.....	15		
do.....	15		

¹ The complete fertilizer used in all three years contained 16.5 per cent N, 16.5 per cent P₂O₅, and 20 per cent K₂O in 1928 and 1929. In 1930 the potash had been increased to 21.5 per cent.

² The source of nitrogen for summer applications in 1928 and 1929 contained 34 per cent N, and 13 per cent lime (CaO).

³ Received for publication Feb. 11, 1932; issued November, 1932. Published as contribution No. 130 of the Massachusetts Agricultural Experiment Station.

⁴ Resigned Oct. 1, 1929.

⁵ Appointed Jan. 2, 1930.

⁶ Grateful acknowledgment is made of the services of M. W. Goodwin, J. W. Kuzmeski, J. B. Zielinski, G. J. Larsinos, and A. F. Spelman, who made the determinations of crude fiber and ether extract under the direction of P. H. Smith, chief chemist of the feed control laboratory of the experiment station; of the services of H. R. DeRose, of the fertilizer control laboratory of the experiment station, who made the soil analyses; and also of the services of C. H. Parsons and R. C. Foley, of the Department of Animal Husbandry, who took practically all of the grass samples. In addition, Mr. Foley has furnished data on botanical analyses of the grasses and his general suggestions have been very helpful. The authors are indebted to A. B. Beaumont, of the Department of Agronomy, for helpful criticism of the manuscript.

⁷ ARCHIBALD, J. G., and NELSON, P. R. THE CHEMICAL COMPOSITION OF GRASS FROM PLOTS FERTILIZED AND GRAZED INTENSIVELY. Jour. Amer. Soc. Agron. 21: 686–699, illus. 1929.

ARCHIBALD, J. G. THE CHEMICAL COMPOSITION OF GRASS FROM PLOTS FERTILIZED AND GRAZED INTENSIVELY IN 1929. Jour. Agr. Research 41: 491–501, illus. 1930.

TABLE 1.—Schedule of fertilizer applications used each year, 1928, 1929, and 1930—Continued

Year, plot group, and treatment	Elements applied	Pounds per acre applied	Dates of application
1929—Continued			
C, no nitrogen; grazed throughout the season.	Phosphoric acid (P_2O_5) ^c	41	May 2.
	Potash (K_2O) ^d	50	Do.
D, control plot.....	No fertilizer.....		
1930			
A and B.....	Nitrogen (N).....	33	Apr. 3-15.
	Phosphoric acid (P_2O_5).....	33	
	Potash (K_2O).....	43	
	Nitrogen (N) ^e	30	May 29-July 9.
	Nitrogen (N).....	20	July 28-Sept. 5.
C.....	Phosphoric acid (P_2O_5).....	33	Apr. 15.
	Potash (K_2O).....	43	Do.
D, control plot.....	No fertilizer.....		

^c As superphosphate.^d As muriate of potash.^e The source of nitrogen for summer applications in 1930 contained 20 per cent N and 20 per cent lime (CaO).

EXPERIMENTAL PROCEDURE

The system of grazing and of taking grass samples has already been described in detail.⁶ It was identical throughout except that in 1930 two of the plots in group A and the control plot (D) were divided in half transversely in order to facilitate uniformity and completeness of grazing.

Except for calcium and phosphorus, methods of chemical analysis were those of the Association of Official Agricultural Chemists.⁷ Calcium was determined volumetrically by McCrudden's method and phosphorus colorimetrically by the method of Fiske and Subbarow.⁸ Botanical analyses of the herbage were made in 1930. The results are given in Table 2. Soil analyses were made in the fall of 1928 on groups A and D only. The results of these analyses appear in Table 3. The soil is a silt loam of the Merrimac series, of level topography. Practically all the 74-acre tract has been cleared, drained, and brought under cultivation within a very few years. Previous to 1928 the tract had been included in a regular farm rotation of corn, 2 years; hay, 2 years; and pasture, 2 or 3 years. The plots in group B were seeded for the first time in 1926.

The composite rainfall and temperature records for the three growing seasons are shown graphically in Figure 1 in comparison with the 40-year normal for Amherst, Mass. The weather was characterized by a normal month of May and a warm, wet June, the other months

⁶ ARCHIBALD, J. G., and NELSON, P. R. Op. cit.⁷ ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. OFFICIAL AND TENTATIVE METHODS OF ANALYSIS. COMPILED BY THE COMMITTEE ON EDITING METHODS OF ANALYSIS. REVISED TO JULY 1, 1924. Ed. 2, 535 p., illus. Washington, D. C. 1925.⁸ FISKE, C. H., and SUBBAROW, Y. THE COLORIMETRIC DETERMINATION OF PHOSPHORUS. Jour. Biol. Chem. 66:387-389. 1925.

having a normal temperature but showing a deficiency in rainfall, which became intensified as the season advanced.

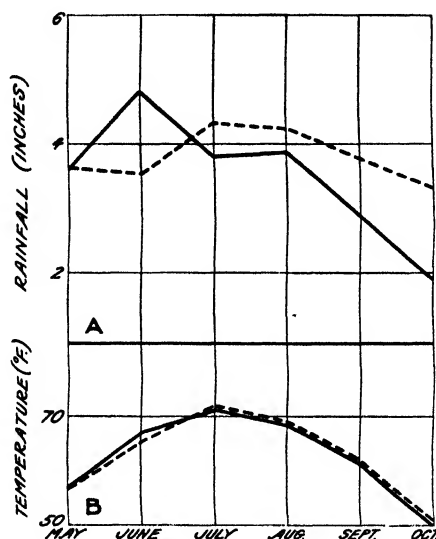


FIGURE 1.—Composite rainfall (A) and temperature (B) records for the growing seasons of 1928, 1929, and 1930. Dotted lines represent normals for Amherst, Mass.

TABLE 2.—Botanical composition of the pasturage herbage on the several groups of plots in 1930

Species	Group A		Group B		Group C		Group D	
	June	August	June	August	June	August	June	August
<i>Phleum pratense</i>	Per cent 21	Per cent 19	Per cent 23	Per cent 25	Per cent 21	Per cent 20	Per cent 7	Per cent 7
<i>Agrostis alba</i>	20	24	39	33	32	37	45	50
<i>Poa pratensis</i>	46	42	27	31	30	25	25	15
<i>Trifolium repens</i>	4	4	1	Trace	7	7	Trace	3
<i>Juncus</i> spp.			3	3				
Other grasses *	5	7			5	5	8	5
Weeds	4	4	7	8	5	6	15	20

* Other grasses included varying amounts of *Dactylis glomerata*, *Anthoxanthum odoratum*, *Lolium italicum*, *Festuca elatior*, and *Poa trivialis*. Weeds included *Taraxacum* spp., *Ranunculus* spp., *Plantago* spp., *Potentilla* spp., *Alisma media*, *Chrysanthemum leucanthemum*, *Rumex acetosa*, *Achillea millefolium*, and *Carex* spp.

TABLE 3.—Results of analyses of soil samples from plot groups A and D, taken in the fall of 1928

Group	Fine soil (1 mm or less)	Organic and volatile matter	Total nitrogen	Total phosphorus	Total potassium	Total calcium	pH *	Lime requirement as CaO, by Jones's method
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent		Pounds per acre
A	98.4	6.63	0.175	0.170	1.17	0.136	5.86	7.136
D	98.0	6.08	.155	.148	1.10	.124	6.25	5.129

* pH determinations were made on samples taken in the spring of 1930. These values were also determined for Groups B and C; they were 5.47 and 5.90, respectively.

PRESENTATION OF RESULTS

EFFECT OF FERTILIZATION AND GRAZING ON THE COMPOSITION OF PASTURE GRASS

The chemical composition of the grass from all groups is shown in Table 4.⁹ The results for Group B have been segregated from those for Group A because the plots in the former group grew a crop of hay each June and were not grazed until July, while those in the latter group were grazed throughout the season each year.

TABLE 4.—*Effect of a system of intensive fertilizing and grazing on the chemical composition (on a dry-matter basis) of pasture grass grown on experimental plots in 1928, 1929, and 1930*

[The values expressed in pounds per acre are true averages of the weights of the crop at the several times of sampling as total weights would not be comparable because of the varying number of samples in the different groups]

Plot group, number of samples, and treatment	Dry matter		Nitrogen		Crude fiber		Ether extract		Calcium		Phosphorus	
	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre
A, average of 73 samples (complete fertilizer plus summer applications of nitrogen).....	27.0	1,054	3.0	31.3	23.4	247	3.3	34.7	0.56	5.9	0.33	3.4
B, average of 39 samples (same fertilizer treatment as above but not grazed until July).....	29.3	954	2.7	25.7	24.8	236	3.2	30.4	.60	5.7	.26	2.4
C (no nitrogen), average of 8 samples (superphosphate, muriate of potash).....	34.2	886	2.4	21.6	23.8	211	3.3	29.5	.60	5.3	.27	2.4
D (control), average of 17 samples (unfertilized).....	34.9	775	2.1	16.1	25.1	195	3.0	23.5	.54	4.2	.29	2.2

Considering the groups in reverse alphabetical order, a study of Table 4 shows that—

There was a gradual but not very uniform decrease in percentage of dry matter, accompanied by a corresponding but more uniform increase in amount of dry matter produced per acre. In other words, one effect of the fertilizer was to produce a considerably larger amount of more succulent grass, even on a dry-matter basis. In Group B, the effect of a hay crop in June with consequent postponement of grazing until July is seen in an increased percentage, and a decreased production, of dry matter. In contrasting C with A and also with D, the effect of a nitrogenous fertilizer in producing a succulent grass and a relatively large amount of dry matter is seen to be very marked.

There was a marked and regular increase in nitrogen content of the dry matter, and an even more marked though less uniform increase in nitrogen recovered per acre. This is the most striking and relatively much the greatest effect of the system, the amount of nitrogen in the samples from Group A being 42.9 per cent greater than in those from Group D, while the amount of nitrogen recovered was almost double (94.4 per cent greater). In Group B, no allowance being made for the hay crop produced in June, the percentage of nitrogen was

⁹ It should be borne in mind in a study of Table 4 and of the discussion which follows that Groups A and D are the two between which fundamental comparisons are made. The results from Groups B and C are included merely for what additional information they may furnish.

28.6 per cent greater than in Group D, while nitrogen recovery was almost 60 per cent more. Group C, which received no nitrogen in 1929 and 1930, evidently had some in reserve in the roots from the application in 1928, being midway between B and D both in percentage and in amount recovered. A quality of herbage superior to that in Group D with somewhat more clover probably accounts for part of the difference.

The crude-fiber content decreased slightly but not uniformly, wherever fertilizer was applied. On the other hand, acre production of fiber increased considerably and quite uniformly. In this latter respect there is quite a close correlation between crude fiber and dry matter. The considerably higher fiber content in Group B than in either A or C is attributed to the presence of stubble from the June hay crop.

The content of ether extract was increased somewhat, and the quantity per acre was increased markedly by the treatment, but neither increase was uniform.

The calcium content of the grass was not materially affected by the fertilizer applications, Group A being only 0.02 per cent higher than Group D, which represents an increase of only 3.7 per cent. Acre recovery was of course considerably increased because of the much larger crop in Group A. The higher calcium content in Groups B and C probably is due to the presence of considerably more white clover (*Trifolium repens*) in the case of Group C, and to much greater amounts of red top (*Agrostis alba*) in both cases, than occurred in Group A. Recent unpublished work by the writers has revealed the fact that red top is somewhat higher in calcium than is either timothy (*Phleum pratense*) or Kentucky bluegrass (*Poa pratensis*). A liberal application of calcium nitrate¹⁰ on the plots in Group B in 1928 is also considered to be a contributing factor.

The percentage of phosphorus was increased considerably in Group A as compared with that in Group D, and the acre recovery was over 50 per cent higher. The low value in Group B is rather difficult to interpret. It was consistently lower from year to year than that in Group D. The explanation offered in the published results for 1929¹¹ is again advanced, to the effect that—

these plots have been brought under cultivation comparatively recently and as a consequence may not have accumulated a phosphorus reserve comparable with that on the first six plots [Groups A and D], which had been fertilized quite liberally over a considerable period of years previous to the commencement of the grazing experiment.

It is also possible that the growing of a hay crop to comparative maturity may account for the disappearance of a considerable share of the store of available phosphorus on these plots. The low value in Group C can not be accounted for on any reasonable basis.

To summarize briefly, the most outstanding effect of the system has been to increase considerably the dry matter produced per acre and at the same time lower the dry-matter content of the fresh grass, and to increase greatly the nitrogen content of the dry matter. All other constituents determined, crude fiber excepted, were increased, although the effect on the calcium content of the grass was slight. Inasmuch as nutritive value of feeds is judged to a considerable extent

¹⁰ For an explanation of the reason for using calcium nitrate, see the following publication: ARCHIBALD, J. G., and NELSON, P. R. Op. cit., p. 638, footnote to Table I.

¹¹ ARCHIBALD, J. G. Op. cit., p. 494.

by a minimum of fiber and a maximum of other proximate constituents, it may be assumed in the absence of actual feeding trials that the grass from Groups A, B, and C was superior as a feed to the grass from Group D, that from Group A being of especially high quality. The grass produced after a crop of hay was somewhat inferior in quality to that produced on plots that were continuously grazed. This is doubtless due in part to the presence of stubble from the hay crop. The effect of withholding nitrogen from Group C naturally was most apparent in the nitrogen content of the samples from that group.

SEASONAL VARIATIONS

As a basis for discussion and for the formulation of general conclusions on this phase of the work, the results from Groups A, C, and D have been combined in Table 5 and are shown graphically in Figure 2. This manner of handling the results was decided upon after it had been observed that in general the fertilizer applications had only a minor influence on the seasonal fluctuations in composition. This being the case, it was felt that by combining the groups the larger number of samples represented would contribute to more reliable final conclusions. Group B was not included because the growth of a hay crop on the plots in this group rendered the values from them not comparable with the others.

In Table 6 the detailed seasonal variations for all the groups are recorded separately, and those for Groups A and D are shown separately in Figure 4, for the sake of comparison with the composite graph in Figure 2 but not as a basis for general discussion. Groups B and C are not represented in Figure 4; the former for the reason given in the preceding paragraph, the latter because the number of samples (eight) when divided among six different months is insufficient to furnish reliable figures.

Figure 1, which gives the composite rainfall and temperature record for the three seasons should be studied in close conjunction with the detailed discussion which follows.

TABLE 1.—Seasonal variations in chemical composition of pasture grass (dry-matter basis); average values for three seasons, 1928, 1929, and 1930

[The values expressed in pounds per acre are true averages of the weights of the crop at the several times of sampling as total weights would not be comparable because of the varying number of samples in the different groups]

Month	No. of samples	Dry matter		Nitrogen		Crude fiber		Ether extract		Calcium		Phosphorus	
		Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre
May.....	20	26.5	608	3.6	21.7	16.9	103	3.6	22.1	0.51	3.1	0.36	2.2
June.....	13	21.7	1,324	2.8	37.2	24.8	328	3.3	43.9	.60	7.9	.36	4.7
July.....	13	27.8	1,170	2.5	29.6	26.5	310	2.9	34.2	.58	6.7	.32	3.8
August.....	19	30.6	1,238	2.6	32.1	26.3	325	3.1	38.1	.67	7.1	.31	3.8
September.....	17	28.7	911	2.8	25.6	24.4	222	3.3	30.5	.56	5.1	.31	2.8
October.....	16	34.8	769	2.6	19.9	21.9	168	3.3	25.7	.59	4.5	.28	2.1
Total.....	98												
Average *		27.7		2.8		24.0		3.2		.57		.32	

* The true average percentage composition of all the samples is recorded here merely as a matter of interest.

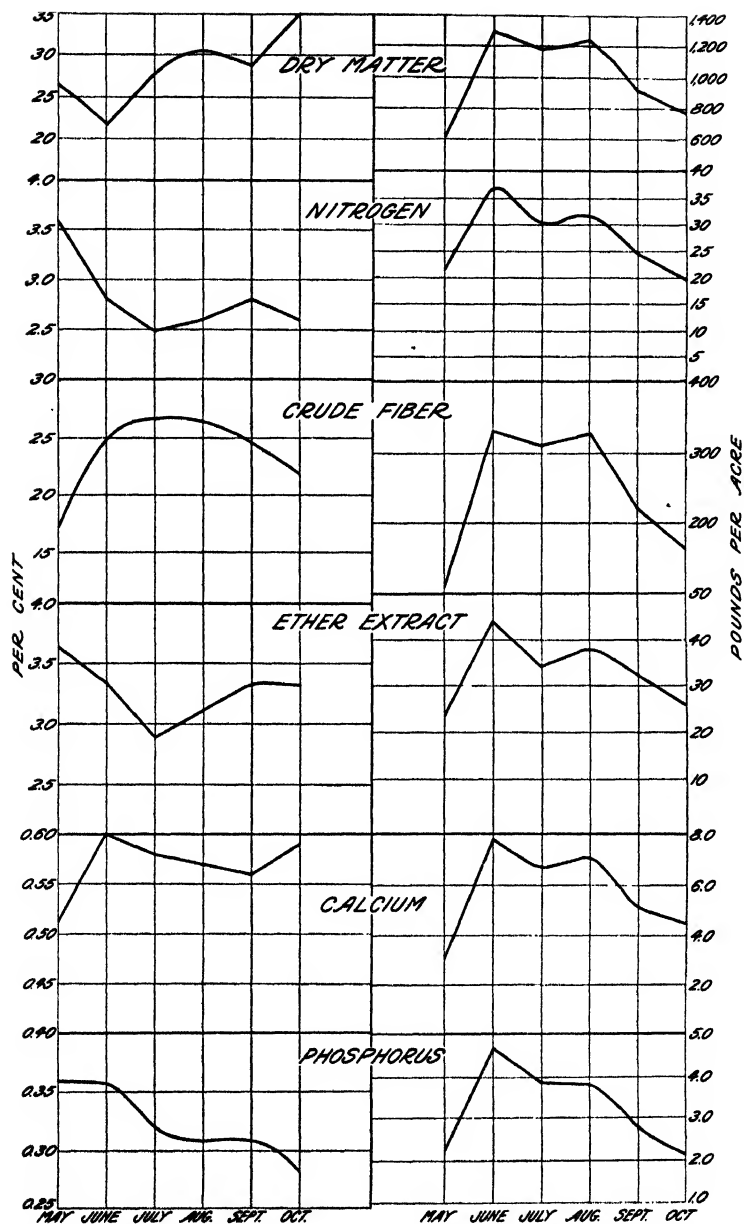


FIGURE 2.—Seasonal variations in the chemical composition of grass from plots fertilized and grazed intensively

DRY MATTER

The curve for the dry-matter content of the grass (fig. 2), shows a general upward trend with the advance of the season. The decrease from May to June is considered to be due largely to the abundant moisture in June, although the presence of dead grass from the previous season's growth may have introduced an error that makes the values for May too high. In view, however, of the drop in nitrogen from May to June and the rise in crude fiber, the possibility of any considerable error in this connection seems remote. The drop in September is thought to be due partly to the late August and September rains and partly to the lower temperatures of autumn which result in less drying out of pastures. In accordance with this reasoning, it would seem that the values for October should be still lower. On the contrary, they are the highest for the season, which is attributed to gradual cessation of growth due to the action of frost.

Comparison of the dry-matter curve with the rainfall curve shows in general a close inverse relationship, although the changes in dry-matter content tend to lag somewhat behind the variations in rainfall.

The curve of dry-matter production has its high point in June with a slightly smaller peak in August. Despite a relatively very low dry-matter content in June, the ideal conditions for growth at that time, viz, abundant moisture and warmth, resulted in the highest dry-matter production of the season. In August the situation was the reverse of that in June—a relatively high dry-matter content offset a much lowered production of grass sufficiently to cause a second peak in the curve. The phenomenal rise in production of dry matter and all its constituents from May to June and the slump in July is clearly depicted in Figure 2.

NITROGEN

The general trend of the curve for nitrogen content of the dry matter is downward with the advance of the season. The marked drop from May to June is particularly worthy of note. It is considered to be due partly to the diminishing influence of heavy early spring applications of nitrogenous fertilizer, but this can not be the only cause as the curve for the control plot which had no fertilizer (fig. 4 (D)), also shows a decrease, although it is much smaller. It has been suggested that root reserves of nitrogen play a part here, being drawn upon extensively for the early spring growth. Also the much greater yield of grass in June than in May probably contributed to the lowered nitrogen content. The considerable rise in nitrogen from July to September is attributed to the influence of the summer applications of nitrogen, as this increase was not manifest in the samples from the control plot. The inverse relationship between nitrogen content and crude-fiber content, except at the end of the season, is very striking.

The curve for nitrogen recovery¹² is of the same general form as that for dry-matter production, as indeed are the production (or recovery) curves for all constituents determined. The form is that of a grotesquely sprawled letter M. In this case the June peak is considerably above the secondary peak in August. The July slump is more marked here than it is in the dry-matter curve. The similarity to the rainfall

¹² The term "recovery" is used instead of "production" in those three cases in which the constituent under discussion is an element (nitrogen, calcium, or phosphorus).

curve (fig. 1) indicates a close relationship between amount of rainfall and nitrogen recovery in the grass.

CRUDE FIBER

The curve for the percentage of crude fiber in the dry matter is the most regular of all. Commencing at much the lowest value in May it rises rapidly to its peak in July, dropping off as regularly but somewhat more slowly as the season wanes. Part of this seasonal fluctuation probably is due to differences in stage of maturity of the samples, but since all samples were taken in the vegetative stage it would seem that there must be other factors of equal or greater importance in this connection. It seems reasonable to ascribe the fluctuations in fiber content largely to the interaction of two important growth factors, viz, moisture and sunshine. So long as the amount and intensity of sunshine is on the increase, which is the case until July, the process of fiber building proceeds rapidly. After June, the increase in fiber is further enhanced relatively by the drying up of other plant tissues which follows in the wake of diminishing moisture supply with the advance of the season. Midsummer or soon thereafter seems to be the peak of the combination or interaction of these two factors, hence the high fiber values for July and August. The decrease in fiber content with the advent of autumn may be due in part to the fact that the samples were less mature than those taken in midsummer, but is ascribed largely to rapid diminution in the amount of sunshine, especially so because the moisture supply remained scanty to the end of the season. This latter fact strengthens the assumption regarding the great influence of sunshine on fiber content for two reasons, (1) if sunshine were not an important factor, the fiber content should increase rather than decrease because of the drying-out effect of an increasing shortage of moisture; and (2) because of the dry autumn, conditions were not favorable for the production of a succulent new growth to which the decreased fiber content might otherwise be attributed.

The principal points of interest brought out by the curve for production of fiber are (1) the very large increase from May to June, which is relatively higher than for any other constituent, and especially high in contrast with nitrogen and ether extract, and (2) the secondary peak in August is of the same magnitude as that for June, while in the case of all other constituents it is somewhat smaller.

ETHER EXTRACT

There is a sharp decrease in the percentage of ether extract from May to July, followed by a somewhat less rapid increase until September. In general the curve resembles that for nitrogen content, and reasons similar to those given for nitrogen fluctuations can be assigned here. That these two constituents are quite closely associated in their ups and downs is further evidenced by the striking similarity of their production curves.

CALCIUM

There is some correlation, although it is by no means close, between calcium content and crude-fiber content. The same marked increase from May to June and the downward trend later is noted in both

cases, although it commences sooner in the case of calcium. It is known that one of the functions of calcium in the plant is as a constituent of the middle lamella of the cell wall, so that a reasonable correlation ought to be evident here. The rise in calcium content in October is probably traceable to the calcium present in summer applications of nitrogenous fertilizers that carried considerable calcium. A study of Figure 4, in which the fertilized and control plot values have been separated, confirms this.

Calcium recovery closely parallels dry-matter production, with a slightly greater peak in June.

PHOSPHORUS

With phosphorus the trend is more definitely downward all through the season than with any other constituent determined. It does not

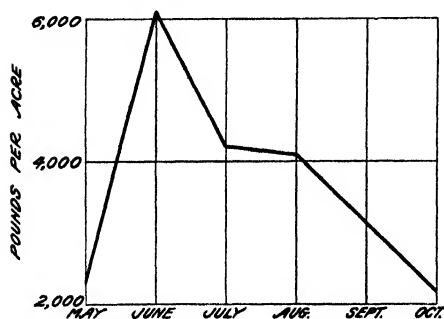


FIGURE 3.—Seasonal variation in production of fresh grass (composite for all plots)

drop so rapidly in the early part of the season as does either nitrogen or ether extract, but the increase later on, which characterizes these, is absent here.

The curve for recovery of phosphorus differs from all others in not having a secondary peak in August, although there is a break in its downward course at that point. Except for the much sharper rise from May to June it resembles the rainfall curve (fig. 1) very closely.

The inference is that in dry seasons there may be a shortage of phosphorus in pastures. This is in agreement with the findings of other investigators.

COMPARISON OF SEASONAL VARIATIONS IN GRASS FROM PLOTS RECEIVING DIFFERENT FERTILIZER TREATMENTS

Considering the seasonal differences in composition as a whole, it is apparent that from the standpoint of quality the grass on these plots was at its best in May, the content of nitrogen, phosphorus, and ether extract being at its maximum then, in association with a minimum of crude fiber and a dry-matter content somewhat above that for June. These facts confirm the old idea that the "early bite" is particularly good feed.

From the standpoint of quantity, the June growth naturally surpassed that for all other months, and except for the fact that it was somewhat more watery, it was comparable in quality with that grown in September, being superior to the September grass in mineral content. The slump in both quantity and quality in July was probably inevitable, but it is interesting to note the improvement in quality which commenced with the passing of midsummer and continued into September. Coupled with a definite increase in August in production of dry matter and of all the constituents except phosphorus, this

improvement indicates that August and September pastures have greater potentialities than are generally recognized. It should be noted, however, that this August increase was due not to an increase in the amount of green grass produced, but to a sharp slackening in

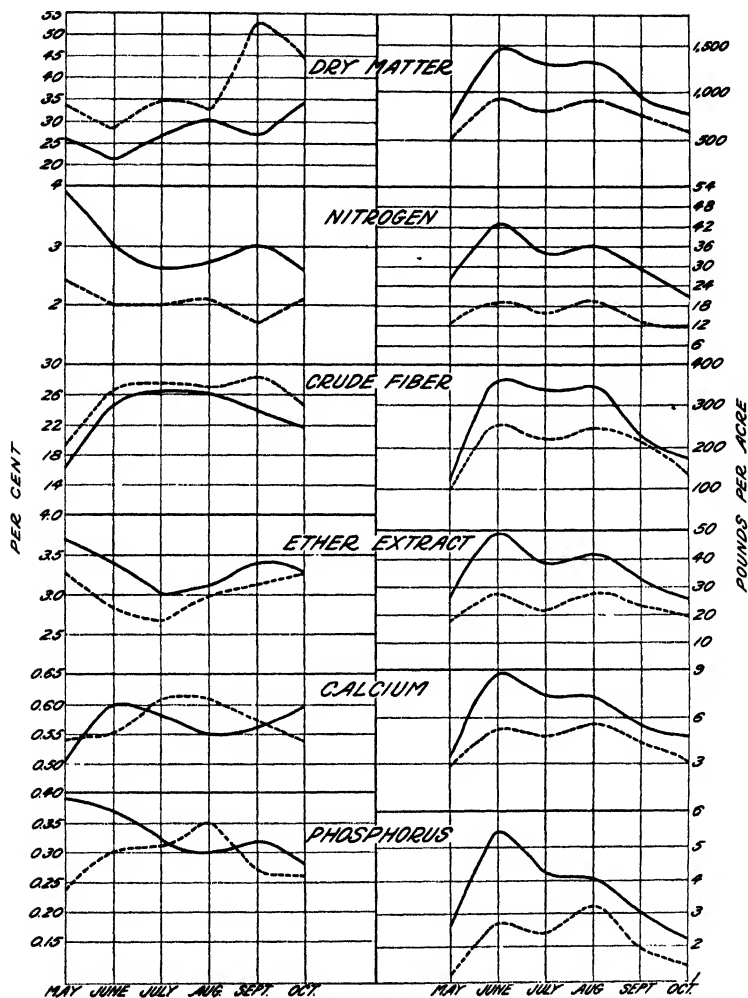


FIGURE 4.—Seasonal variations in the composition of grass from fertilized and from unfertilized plots, groups A and D only. Group A, solid line; Group D (control), broken line

the rate of decrease in production of green grass (fig. 3), accompanied by a relatively high dry-matter content.

Although in the nature of things uniform production and quality throughout the season are impossible of attainment, it is believed that some of the irregularities could be smoothed out considerably. If some of the June peak could be pushed over into the July depression

and if the relatively high quality of September grass could be attained a month earlier, one of the difficulties of pasture management would be minimized considerably. Less fertilizer in the spring and more in the summer would be a step in this direction, if summer labor conditions permitted such a change of plan. The problem is one for the agronomist and farm-management specialist.

TABLE 6.—Seasonal variations in chemical composition (on a dry-matter basis) of pasture grass grown on plots fertilized with and without nitrogen, and on check plots, in 1928, 1929, and 1930

Plot group, month, and treatment	Dry matter		Nitrogen		Crude fiber		Ether extract		Calcium		Phosphorus	
	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre	Per cent	Pounds per acre
A (Complete fertilizer plus summer applications of nitrogen):												
May.....	25.1	665	3.9	25.9	16.2	108	3.7	24.7	0.50	3.3	0.39	2.6
June.....	20.7	1,450	3.0	43.2	24.6	357	3.4	49.2	.60	8.7	.37	5.4
July.....	26.8	1,205	2.6	33.2	20.4	333	3.0	37.3	.58	7.3	.32	4.1
August.....	29.5	1,325	2.7	35.9	26.1	346	3.1	41.5	.55	7.3	.30	4.0
September.....	26.0	942	3.0	28.7	23.8	224	3.4	31.9	.56	5.3	.32	3.0
October.....	34.3	791	2.6	20.8	21.6	171	3.3	25.9	.60	4.7	.28	2.2
B (Same fertilizer as above but not grazed until July):												
May.....	23.6	711	3.8	27.3	19.0	135	3.6	25.3	.55	3.9	.34	2.4
June.....	26.8	1,149	2.6	28.5	27.1	311	3.0	34.6	.67	7.6	.27	3.1
July.....	32.5	1,049	2.5	26.1	26.4	277	3.0	31.6	.56	5.8	.25	2.6
August.....	30.2	880	2.5	21.6	24.8	154	3.3	29.2	.54	4.8	.24	2.1
September.....	38.0	863	2.4	20.4	23.2	200	3.3	28.5	.62	5.3	.21	1.8
C (No nitrogen, superphosphate, muriate of potash):												
May.....	32.6	416	2.7	11.2	18.6	78	3.6	14.8	.49	2.1	.30	1.3
June.....	22.0	1,323	2.9	38.6	21.7	287	3.7	48.4	.70	9.2	.32	4.3
July.....	34.4	976	2.2	21.9	26.7	261	3.0	29.0	.50	4.9	.33	3.2
August.....	47.6	1,352	2.2	30.2	26.4	357	2.7	35.8	.73	9.8	.22	3.0
September.....	45.0	867	2.1	17.9	25.2	219	3.3	28.8	.54	4.7	.24	2.1
October.....	32.0	871	2.7	23.6	21.9	191	4.1	35.9	.57	5.0	.25	2.2
D (Control) unfertilized:												
May.....	33.0	506	2.4	12.2	19.0	96	3.3	16.6	.54	2.8	.24	1.2
June.....	28.1	946	2.0	18.6	26.8	254	2.8	26.7	.55	5.2	.30	2.8
July.....	34.4	790	2.0	15.7	27.7	219	2.7	21.4	.61	4.8	.31	2.4
August.....	32.2	904	2.1	19.3	26.9	244	3.0	27.0	.61	5.5	.35	3.2
September.....	53.3	756	1.7	12.9	28.5	215	3.1	23.1	.57	4.3	.27	2.1
October.....	44.1	575	2.1	11.8	24.3	140	3.3	19.2	.54	3.1	.26	1.5

* Cut for hay.

Attention is directed briefly to Table 6 and Figure 4. In the latter a comparison is drawn between the values obtained on the fertilized plots and those obtained from the control plot. In general the curves are quite similar in trend. The high point of production in June was accentuated by the use of fertilizer, but the high point in August (phosphorus excepted) was of similar magnitude in both cases. The tendency for the curves to approach each other at each end of the season while being relatively well separated from June to September, is worthy of note.

It is interesting to observe the relatively great difference in both composition and production between the two groups in May as regards nitrogen, phosphorus, and ether extract.

The curves for content of calcium and phosphorus are an exception to the general similarity in the fluctuations of the two groups. One

effect of the fertilizer seems to have been to speed up assimilation of calcium early in the season with the result that the highest value and the subsequent decrease came about a month earlier than they did on the check plot. The second rise in the curve for fertilized plots from August onward is attributed, as already stated, to the readily soluble calcium contained in summer applications of nitrogenous fertilizers carrying considerable calcium. It is thought that the curve for the check plot more nearly represents the normal seasonal changes in calcium content. It is in agreement with the curves for calcium given by Orr.¹³

A similar stimulating effect of the fertilizer on phosphorus assimilation is evident, the high point for phosphorus content coming in May where fertilizer was applied, but not until August on the control plot.

SUMMARY AND CONCLUSIONS

The results of analyses of 137 samples of grass taken during the growing seasons of 1928, 1929, and 1930 from nine 8-acre pasture plots which were fertilized and grazed intensively are reported, discussed, and interpreted. The fertilizer treatment varied slightly from season to season, but the general plan involved the application of a concentrated complete fertilizer in early spring, followed by several summer applications of a concentrated nitrogenous fertilizer. The plots were grazed in rotation and stocked with sufficient cattle to keep the grass quite short. Samples of the herbage on each plot were taken at the commencement of the pasture season in May, and at the conclusion of the rest periods of the plots, just before the cattle were turned in. Dry matter, total nitrogen, crude fiber, ether extract, calcium, and phosphorus were determined.

The most outstanding effects of the system were to increase considerably the dry matter produced per acre and at the same time to lower the dry-matter content of the fresh grass, and to increase greatly the nitrogen content of the dry matter. All other constituents, crude fiber excepted, were increased, although the effect on the calcium content of the grass was slight. The effect of nitrogen in producing a succulent grass and a relatively large amount of dry matter was very marked.

Inasmuch as the nutritive value of feeds is measured somewhat by a minimum of fiber and a maximum of other constituents, it may be assumed, in the absence of actual feeding trials, that the grass from the fertilized plots was superior as a feed to that from the control plot.

A study of the seasonal variations in 98 of the samples showed that the quality of the grass on these plots was at its best in May, and that in quantity of grass, June surpassed all the other months. Except in its mineral content, the September grass was comparable in quality with that grown in June.

The decrease in production in July while very evident was followed by just as evident an increase in August, some constituents showing nearly as high a production then as they did in June. This August increase was in dry matter, not in fresh grass.

Except in the case of the minerals (Ca and P), the assimilation of which it evidently stimulated, the fertilizer treatment had little effect

¹³ Orr, J. B., with the assistance of SCHERRATOFF, H., MINERALS IN PASTURE & THEIR RELATION TO ANIMAL NUTRITION. P. 38-39. London. 1920.

on seasonal fluctuations in composition. It did, however, accentuate markedly the peak of production in June.

There is a close correlation between amount of rainfall and production or recovery of the more valuable constituents of the grass. This is especially true of the nitrogen, phosphorus, and ether extract.

Grass kept in the vegetative stage by grazing may be quite different in chemical composition in midsummer from what it was in the spring. The growth factors, notably the amount of rainfall and sunshine, exert their influence irrespective, to some extent at least, of the stage of growth of the plant.

The practical suggestion which is the obvious corollary to this study is that an attempt should be made to lessen these seasonal irregularities if and where possible. Less fertilizer in the spring and more in the summer is suggested as one remedy, if it be practicable.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 45

WASHINGTON, D. C., DECEMBER 1, 1932

No. 11

COMPARISON OF CONFORMATION, ANATOMY, AND SKELETAL STRUCTURE OF THE COW AND BULL OF A DAIRY BREED¹

By W. W. SWETT, *Senior Dairy Husbandman*; R. R. GRAVES, *Chief*; and FRED W. MILLER, *Senior Veterinarian and Physiologist, Division of Dairy Cattle Breeding, Feeding, and Management, Bureau of Dairy Industry, United States Department of Agriculture*

INTRODUCTION

A comparison of the conformation, anatomy, and skeletal structure of two cows representing the highly specialized dairy and the highly specialized beef types was presented in a previous paper.² Although these cows were found to differ greatly in external form, the differences in weight and size of their internal organs were not sufficient to indicate significant differences in function, and their skeletal structure was similar in most particulars. Except for the extreme differences in external form, the quantity of secretory tissue in their udders was the most striking difference noted. These findings led to the conclusion that the evolution of the dairy and beef types, which has been accomplished through breeding and selection over a period of many years, has not materially altered their skeletal structure, but that the difference in type is due to extreme fleshing on the one hand and to udder development and absence of fleshing on the other. The results of this study suggested the advisability of making a similar comparison of the conformation, anatomy, and skeletal structure of a cow and a bull of the same breed.

Sophie 19th of Hood Farm 189748, whose anatomical data had already been completed and published, was chosen for this study. She had made a world's record by producing 17,557.8 pounds of milk and 999.1 pounds of butterfat in one year. She had also made a world's record for lifetime production by producing 7,544.51 pounds of butterfat in 11 yearly official records. The bull used for comparison was Majesty's Fern's Noble Fox 193952, a representative specimen of the Jersey breed. For the sake of brevity, Sophie 19th of Hood Farm and Majesty's Fern's Noble Fox will subsequently be referred to as "the cow" and "the bull."

The average weights of Jersey cows and bulls are about 900 pounds and 1,500 pounds, respectively.³ When the cow was measured she was in fair flesh and weighed 927 pounds. She would probably be considered somewhat above the average for the breed in size—especially in frame. The bull was in good flesh and weighed 1,500 pounds

¹ Received for publication Mar. 9, 1932; issued November, 1932.

² SWETT, W. W., GRAVES, R. R., and MILLER, F. W. COMPARISON OF CONFORMATION, ANATOMY, AND SKELETAL STRUCTURE OF A HIGHLY SPECIALIZED DAIRY COW AND A HIGHLY SPECIALIZED BEEF COW. *Jour. Agr. Research* 37: illus. 685-717. 1928.

³ NYSTROM, A. B. DAIRY CATTLE BREEDS. U. S. Dept. Agr. Farmers' Bull. 1443, p.26. 1930.

when the ante-mortem data were obtained. He can, therefore, be considered as of average size for the breed. The cow was 19 years and 4 months of age and the bull was 6 years and 6 months of age. Photographs of the living animals are shown in Figures 1-3.

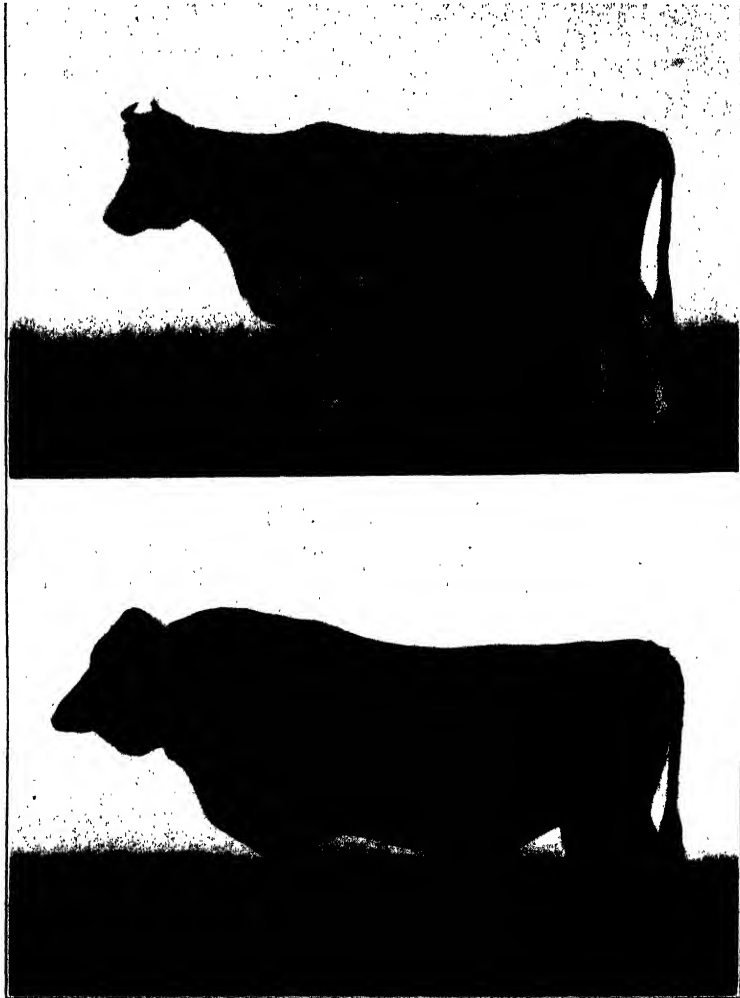


FIGURE 1.—Side view of cow and bull

ANTE-MORTEM DATA

The conformation or type of an animal must be expressed in numerical terms before it can be analyzed or compared with that of another. This is accomplished by making a number of external measurements, so selected that they represent the size, proportions, and outlines of the body. The method of measuring adopted by the



FIGURE 2.—Front view of cow and bull



FIGURE 3.—Rear view of cow and bull

Bureau of Dairy Industry for studying growth, and the relationship between conformation, anatomy, and producing capacity of dairy cows, was applied in making this comparison. The difficulties experienced in measuring the Aberdeen Angus cow⁴ were not encountered in the case of this bull as he was not carrying an excessive amount of flesh. The ante-mortem data in this comparison are, therefore, more nearly representative of the actual conformation of the animals, since the body landmarks were readily located. The external body measurements which represent the conformation of the cow and the bull, together with the relation of the measurements of the bull to those of the cow, expressed in percentage, are given in Table 1.

TABLE 1.—*Ante-mortem external measurements of cow and bull*

Item measured	Instrument used	Unit	Cow	Bull	Relation of measurements of bull to those of cow
					<i>Per cent</i>
Thickness of hide	Vernier caliper	Centimeters	0.66	1.16	175.8
Live weight		Pounds	927.00	1,500.00	161.8
Height at withers	Measuring standard	Centimeters	128.17	128.06	99.9
Height at hips	do	do	122.50	125.31	102.3
Height at pin bones	do	do	117.00	121.50	103.8
Length, top hips to top pin bones (length of rump)	Tape line	do	41.00	42.50	103.7
Depth of fore chest	Caliper	do	71.50	73.50	102.8
Depth of rear chest	do	do	71.00	76.00	107.0
Depth of paunch	do	do	71.17	80.00	112.4
Width of fore chest	do	do	36.17	50.00	138.2
Width of rear chest	do	do	56.33	67.00	118.9
Width of paunch	do	do	60.17	73.50	122.2
Width of hips	do	do	48.00	50.25	104.7
Width of pin bones	do	do	29.50	32.00	108.5
Width of thurls	do	do	43.00	44.50	103.5
Width of loin	do	do	29.00	32.50	112.1
Length, withers to line between hips	Tape line	do	88.50	92.00	104.0
Length, line between hips to pin bones	do	do	53.00	51.00	96.2
Total length from withers to pin bones	do	do	141.50	143.00	101.1
Length of loin	do	do	38.00	37.00	97.4
Circumference of fore chest	do	do	177.67	209.00	117.6
Circumference of rear chest	do	do	203.67	229.00	112.4
Circumference of paunch	do	do	213.67	249.00	116.5
Width of forehead	do	do	22.00	29.00	131.8
Circumference of muzzle	do	do	44.00	56.00	127.3
Length from poll to mouth	do	do	56.00	61.00	108.9
Circumference of shin bone	do	do	16.50	19.00	115.2

The percentage value in each instance shows the relation of the measurement of the bull to that of the cow. A value above 100 indicates a greater measurement for the bull than for the cow, and a value below 100 indicates a greater measurement for the cow than for the bull. Comparing the conformation of the two animals on the basis of these percentage values is more significant than comparing them on the basis of the difference in units of measurement, for a difference of 1 cm in one body measurement may be proportionately as great as a difference of 10 to 15 cm in some other measurement. For example, a difference of 2.5 cm in circumference of

⁴ SWETT, W. W., GRAVES, R. R., and MILLER, F. W. Op. cit.

shin bone gives a percentage of 115.2, whereas a difference of 35.33 cm in circumference of paunch gives a percentage of 116.5.

The highest percentages are for thickness of hide and live weight, neither of which is indicative of conformation. Of the 25 measurements which are indicative of skeletal size, body proportions, or conformation, the one having the highest percentage is width of fore chest and the one having the lowest percentage is the length from a point midway between the hips to the pin bones.

Five groups of items representing (1) lengths, (2) heights, (3) depths, (4) widths, and (5) circumferences have been studied separately.

Percentages for the measurement of length range from 108.9 to 96.2, and average 101.9 when the length of head (poll to mouth) is included and 100.5 per cent when omitted. This shows that the two animals differed very little in length.

The three percentages for height range from 103.8 to 99.9 and average 102.0, showing that in height of body also the two animals were nearly the same.

The percentages for depth of body range from 112.4 to 102.8 and average 107.4, indicating that the depths of the two animals differed relatively more than either the lengths or heights, but were not extreme.

Body widths are not entirely consistent in their relationships. The percentages range from 138.2 to 103.5. The average of the eight which constitute this group is 117.5, but if the width of forehead is excluded the average is reduced to 115.4. Three (width of fore chest, rear chest, and paunch) apply to barrel measurements. They range from 138.2 to 118.9 and average 126.4. Four of the widths (loin, pin bones, hips, and thurls) are measurements of body dimensions but are more significant of skeletal width than are the three barrel widths. The range for these four skeletal widths is from 112.1 to 103.5 per cent and the average is 107.2 per cent. In relative width of body the bull was, therefore, considerably greater than the cow.

Five circumferences were measured. The percentages range from 127.3 to 112.4 and average 117.8. The exclusion of circumference of muzzle and circumference of shin bone reduces this average to 115.5. The circumferences of the bull are, therefore, distinctly greater than those of the cow, and represent the highest average percentage of any group.

The average of the percentages for the entire group of 25 body measurements is 110.7, which shows that the bull, in general, was larger than the cow but that the difference in measurements does not approach the difference in live weight. This is true not only of the averages for any group but for every individual item measured. The exclusion from group averages, of length from poll to mouth, width of forehead, circumference of muzzle, and circumference of shin bone, has been made because in some respects these items may not be as indicative of body size or body scale as the others.

As a supplement to the data in Table 1, a number of additional items were derived in order to determine more completely the relation between the conformation of the cow and of the bull. The results are given in Table 2.

TABLE 2.—*Supplemental ante-mortem data and factors showing relation between conformation of cow and bull*

	Cow	Bull	Relation of conformation of bull to conformation of cow
Contour area of fore chest:			<i>Per cent</i>
Total..... square centimeters.....	1,882	2,762	146.8
Portion above vertical mid-point..... do.....	973	1,416	145.5
Portion below vertical mid-point..... do.....	909	1,346	148.1
Percentage of total above vertical mid-point..... per cent.....	51.7	51.3	
Percentage of total below vertical mid-point..... do.....	48.3	48.7	
Contour area of paunch:			
Total..... square centimeters.....	3,226	4,559	141.3
Portion above vertical mid-point..... do.....	1,425	2,018	141.6
Portion below vertical mid-point..... do.....	1,801	2,541	141.1
Percentage of total above vertical mid-point..... per cent.....	44.2	44.3	
Percentage of total below vertical mid-point..... do.....	55.8	55.7	
Slope of rump..... degrees-minutes.....	-7 43	-5 9	66.7
Thoracic index..... ratio.....	1.977	1.470	74.4
Abdominal index..... do.....	1.183	1.088	92.0
Volume of barrel..... cubic centimeters.....	226,029	336,766	149.0
Body-surface area..... square centimeters.....	47,470	57,012	122.0
Legginess..... ratio.....	0.442	0.426	96.4
Wedge shape:			
Difference in measurements—			
Depth of paunch minus depth of fore chest..... centimeters.....	-0.33	+6.50	
Width of paunch minus width of fore chest..... do.....	+21.00	+23.50	
Circumference of paunch minus circumference of fore chest..... centimeters.....	+36.00	+40.00	
Contour area of paunch minus contour area of fore chest..... square centimeters.....	+1,344	+1,797	
Ratio of measurements—			
Depth of paunch to depth of fore chest..... ratio.....	0.905	1.088	109.3
Width of paunch to width of fore chest..... do.....	1.664	1.470	88.3
Circumference of paunch to circumference of fore chest..... do.....	1.203	1.191	99.0
Contour area of paunch to contour area of fore chest..... do.....	1.714	1.651	96.3

At the time the ante-mortem measurements were made with tape line, measuring standard, and calipers, contours or cross-section outlines of the body at the fore chest and paunch were drawn life-size on specially ruled sheets of paper. An apparatus designed and constructed for the purpose was used in making the contours. In many respects contours are more significant than caliper measurements, for two animals that have identical vertical and transverse measurements may differ greatly in cross-section areas as a result of differences in shape. For example, the chest of one may be full and rounded whereas that of another may be constricted above or below the point of maximum width. Again, the paunch of one may be round and full with the maximum width near the vertical mid-point, whereas that of another may be "slab-sided," with its maximum width near the lower extremity of the vertical axis. The areas of the contours were measured with a planimeter. A study of a large number of body contours throws some light on the distribution of the cross-section area of the body at different planes. The writers expect to study the relation of that distribution to the size of some of the internal organs. The contours of the fore chest of the cow and bull are illustrated in Figure 4; those of the paunch are shown in Figure 5.

Both of the percentages showing the relation between the total contour area of the bull and that of the cow at the fore chest and at the paunch (146.8 and 141.3, respectively) are higher than any of those representing body conformation in Table 1. To determine the distribution of a contour area, the procedure regularly followed

is to divide each contour into two parts by drawing a horizontal line through a point on the median line exactly halfway from its highest to its lowest extremity. The two areas formed by the horizontal line through the vertical mid-point are measured separately and are referred to as the portions above and below the vertical mid-point. Although wide variations in the distribution of the areas of contours have been found to occur with other animals of the same breed, the distribution for fore chest and for paunch is remarkably similar for the cow and bull. In the fore-chest contours the portions above and below the vertical mid-point were, respectively, 145.5 and 148.1

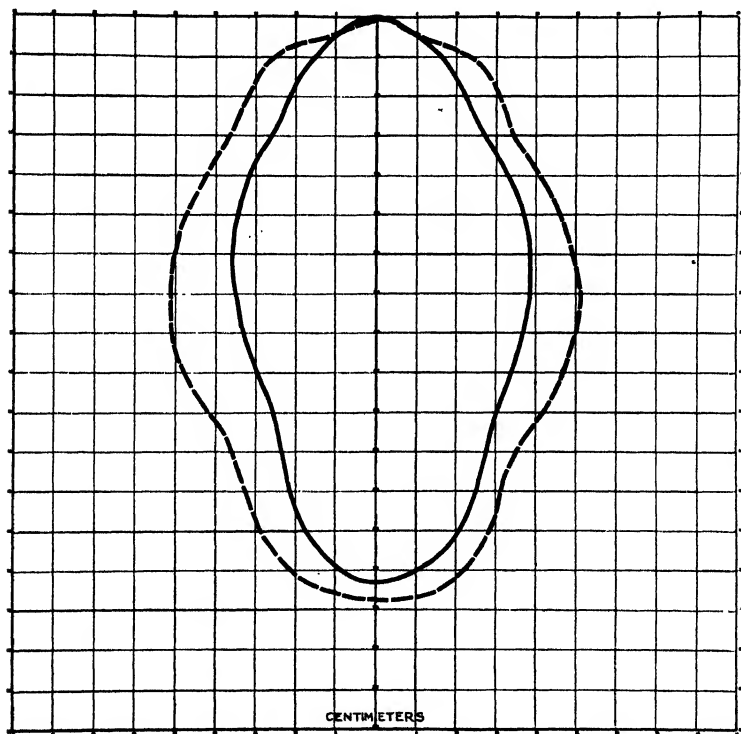


FIGURE 4.--Contours of fore chest (cow, solid line; bull, broken line)

per cent as great for the bull as for the cow. Both of these percentages are similar to the percentage (146.8) representing the relationship between the total fore-chest areas. Also, the portion of the total area above the vertical mid-point was 51.7 per cent for the cow and 51.3 per cent for the bull.

The similarity of distribution of areas in the fore-chest contours of the two animals is shown in every comparison in spite of the fact that the two contours differ considerably in shape. (Fig. 4.) The contour areas of the paunch also differ in shape, although both indicate a deep but slab-sided barrel. (Fig. 5.) As in the case of the fore chest, both of the percentages which show the relation of bull to cow

for the portions above and below the vertical mid-point are very close to the corresponding one for total paunch areas. Although slightly more than half the total area of the fore chest was above the vertical mid-point, considerably more of the paunch was below the vertical mid-point in both the cow and the bull. This is in accord with observations that both animals were deep in the paunch and inclined to be slab-sided. The distribution of paunch areas for the cow and bull is surprisingly similar.

The slope of rump, or its angle of inclination from the horizontal, was calculated on the basis of the difference between the height at

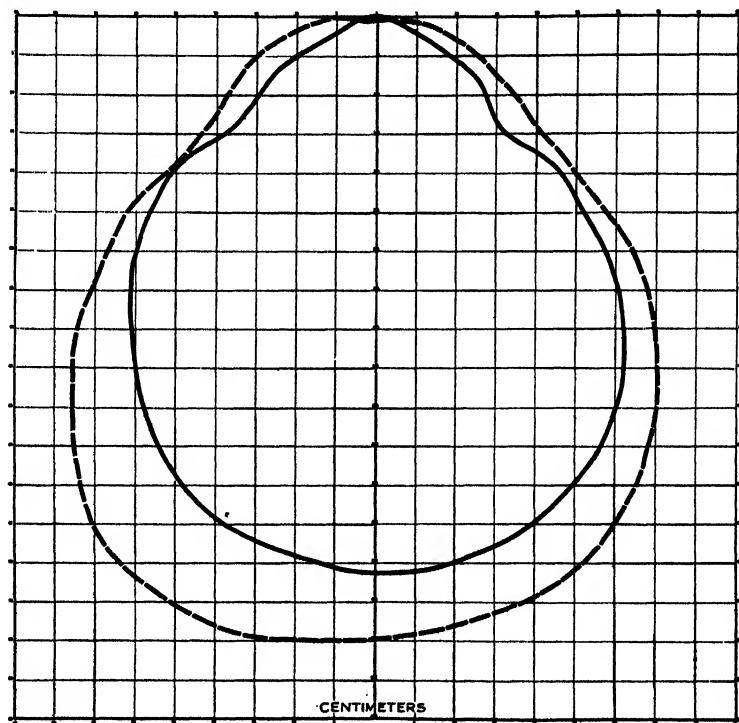


FIGURE 5.—Contours of paunch (cow, solid line; bull, broken line)

hip and the height at pin bone and the linear distance between these two points. When the pin bone is lower than the hip point the angle of rump is minus; when the pin bone is higher than the hip point it is plus. The angle of inclination, or slope of rump, of the bull was $-5^{\circ} 9'$ as compared to $-7^{\circ} 43'$ for the cow. The percentage showing the relation of the slope of rump of the bull to that of the cow (66.7) is distinctly misleading, as it indicates a wide difference between the two animals. However, in consideration of the fact that the angles of rump measured in the writers' studies with a large number of animals have varied from $+9^{\circ} 36'$ to $-18^{\circ} 58'$, a total range of $28^{\circ} 34'$, the difference in angle between the cow and bull is almost negligible.

The thoracic index is the relation of depth to width of fore chest. The thoracic indexes indicate that the fore chest of the cow is relatively deep and narrow, whereas that of the bull is decidedly wider in proportion to its depth.

The abdominal index is the relation of depth to width of paunch. The abdominal indexes show that the bull was only slightly wider than the cow in proportion to depth of paunch.

Volume of barrel is an approximation obtained by multiplying one-half the sum of the fore-chest and paunch-contour areas by the length from withers to a line between the hips. The volume of the bull was 149 per cent as great as that of the cow. This percentage is similar to those for contour areas of fore chest and paunch, and is higher than any other percentage showing the relation between the measurements of the bull and the cow, except thickness of hide and live weight, neither of which is indicative of skeletal size, body proportions, or conformation.

The body-surface area was calculated by the Hogan and Skouby⁵ formula ($S = W^{.4} \times L^{.6} \times K$), in which S =surface of body in square centimeters, W =live weight in kilograms, L =length from withers to pin bones in centimeters, and K =a constant, which for cattle is 217. The area of the bull was 122 per cent as great as that of the cow, which is near the maximum of the percentages representing the relation of the conformation of the bull to that of the cow, and higher than the average for any major or minor group of measurements except the average for the three widths of barrel (126.4 per cent) taken at the fore chest, rear chest, and paunch.

Legginess is the relation of length of forelegs to height at withers and is determined by dividing the length of forelegs by the height at withers. Length of forelegs is determined by subtracting the depth of fore chest from the height at withers. The two animals did not differ greatly in legginess.

The wedge shape of an animal is the amount of divergence between the opposite walls of the barrel. It is determined by comparing the measurements of the paunch with those of the fore chest and is expressed in two ways: (1) The difference in units of measurement between the depths, widths, circumferences, and contour areas of the paunch and fore chest, and (2) the ratio of the depth, width, circumference, and contour area of the paunch to the depth, width, circumference, and contour area of the fore chest. The two animals differed considerably in wedge shape. The cow was slightly deeper at the chest than at the paunch, but the bull was 6.50 cm deeper at the paunch than at the chest, showing that the vertical wedge of the bull was decidedly greater than that of the cow. Other differences in wedge shape are shown in Table 2.

Since the bull was much larger and heavier than the cow, one might expect a greater difference in units of measurement between the paunch and fore chest of the bull than between those of the cow. However, the ratios of the paunch measurements to the fore-chest measurements of depth, width, circumference, and contour areas for the cow and bull may be independent of the size of the animal, provided the distances between the planes used in measuring the fore chest and paunch of the cow and the bull do

⁵ HOGAN, A. G., and SKOUBY, C. I. DETERMINATION OF THE SURFACE AREA OF CATTLE AND SWINE. Jour. Agr. Research 25 : 419-430, illus., 1923.

not differ materially. The average for all the length measurements of the body, except the length of the head, is 100.5 per cent and the length from withers to hips is only 104.0 per cent as much for the bull as for the cow. These differences are among the lowest recorded for any of the body measurements.

As an illustration of the relative significance of the differences in units of measurement between paunch and fore chest and of the ratios between these measurements, let it be assumed that one animal measures 20 cm in width of fore chest and 30 cm in width of paunch, while another measures 40 cm in width of fore chest and 60 cm in width of paunch. The difference in units of measurement would be 10 for the former and 20 for the latter. On this basis the value for the second animal would be 200 per cent as great as for the first. On the basis of ratios of paunch to fore chest, each would be 1.50 and the relation of one to the other would be 100 per cent. The differences in units of measurement are, therefore, less significant of the relative wedge shape of the two animals than are the ratios of the paunch to the fore-chest measurements. Because of this fact the percentages showing the relation of the conformation of the bull to that of the cow are omitted for the differences but are included for the ratios. The differences, however, show very effectively the "reverse wedge" in depths for the cow, and that, although the bull is much larger than the cow, the difference between widths of paunch and fore chest is slightly greater for the cow than for the bull. This undoubtedly is due to the fact that the cow was deep and narrow in the fore chest and the bull was relatively much wider in proportion to his depth. This observation is in agreement with the thoracic indexes reported. Although differences in measurements would give one the impression that, except for width, the bull had greater wedge shape than the cow, the ratios themselves and the percentages indicate that the wedge shape of the bull was less than that of the cow in every measurement except depth.

POST-MORTEM DATA

A post-mortem study of the anatomy of the cow and the bull was made in accordance with the plan adopted by the Bureau of Dairy Industry for the purpose of determining the relation of conformation and anatomy to milk and butterfat producing capacity. The cow was killed by the injection of chloroform and the bull was killed by bleeding. The cow's lungs and liver retained large quantities of coagulated blood, so that these organs were excessive in weight and could not be compared satisfactorily with those of another animal. The bull's organs, when weighed, were all practically free of blood. The necessity for preserving the skeleton without injury prevented the recovery of the brain and pituitary body. The weights and measurements of the organs recovered which were not materially affected by blood retention, are listed for comparison in Table 3.

The term "empty body weight" is used in this study to represent the net weight of the total animal structure. It is determined by subtracting the total weight of the contents of stomachs and intestines from the live weight. Empty body weight is more significant than live weight because it eliminates variations due to differences in amount of feed or water consumed before weighing or to differences in intervals between feeding or watering and time of weighing.

TABLE 3.—Post-mortem data for cow and bull

Item	Weight or measurement of organ or part				Relation of data of bull to those of cow	
	Cow		Bull		Actual units	Units per 100 pounds empty body weight
	Actual units	Units per 100 pounds empty body weight	Actual units	Units per 100 pounds empty body weight		
Empty body weight	pounds	768.50	1,329.30	173.0	Per cent	Per cent
Weight of hide	do	55.00	115.00	8.65	209.1	120.8
Weight of pancreas	grams	(a)	640.00	4.81	-----	-----
Weight of kidneys	do	1,445.85	1,270.03	95.55	87.8	50.8
Weight of adrenals	do	37.00	41.40	3.11	111.9	64.7
Weight of spleen	do	822.15	1,542.24	116.02	187.6	108.5
Weight of liver	pounds	(b)	20.60	1.55	-----	-----
Weight of small intestine	do	13.00	15.65	1.18	120.4	69.8
Weight of large intestine	do	22.50	10.70	.80	47.6	27.3
Total weight of empty intestines	do	35.50	26.35	1.98	74.2	42.9
Weight of intestine contents	do	32.00	20.90	1.57	65.3	37.7
Length of small intestine	feet	132.50	104.98	12.41	124.5	72.0
Length of large intestine	do	34.00	41.98	3.16	123.5	71.5
Total length of intestines	do	166.50	206.96	15.57	124.3	71.9
Total weight of empty stomachs	pounds	47.00	37.05	2.79	78.8	45.6
Weight of stomach contents	do	126.50	93.80	7.06	74.2	42.9
Weight of abdominal fat	do	34.63	63.15	4.75	182.4	105.3
Weight of right lung	do	(c)	4.80	.36	-----	-----
Weight of left lung	do	(c)	3.90	.29	-----	-----
Total weight of lungs	do	(c)	8.70	.65	-----	-----
Circumference of heart (near base)	centimeters	45.00	45.50	3.42	101.1	58.4
Circumference of heart (over apex)	do	48.00	54.50	4.10	113.5	65.6
Weight of heart (auricles removed)	pounds	3.94	4.75	.36	120.6	70.6
Weight of thoracic fat	do	5.50	3.80	.29	69.1	40.3
Weight of thyroid	grams	23.00	72.00	5.42	313.0	181.3

* Pancreas omitted.

b Liver abnormal.

c Lungs filled with clotted blood.

Table 3 gives for the cow and the bull (1) the weight or measurement for each item, (2) the number of units of weight or measurement for each 100 pounds of empty body weight, and (3) the percentage relation of the anatomy of the bull to that of the cow on the basis of both. Twenty different items are compared. In 13 of these the weight or measurement for the bull is greater. In the group of 7 items which weighed or measured less for the bull than for the cow, 2 represent contents of the digestive tract which are subject to conditions of management and which consequently have little significance; 1 is the weight of thoracic fat, which probably is also of little significance; 2 are weights of empty intestines, which may be affected to a considerable extent by inability to remove all of the fat, and 2 are total weight of empty stomachs and weight of kidneys, both of which are readily prepared and comparable.

Although the weight of intestines is subject to some error as a result of adhering fat, the length of intestines can be measured readily and accurately. The length of the cow's intestine does not approach the maximum or the minimum recorded in a previous post-mortem study of many cows representing several breeds of dairy cattle.⁶ The length of the bull's intestine, although considerably greater than that of the cow, is about 35 feet less than the maximum recorded in the study mentioned.

⁶ Unpublished data in files of Bureau of Dairy Industry.

The retention of blood in the lungs of the cow prevented a determination of the total lung weight. The total lung weight of the bull was 8.70 pounds. For 229 cows slaughtered in a packing house, whose average empty body weight was 941 pounds, the average lung weight was 7.36 pounds. This is 0.78 pound for each 100 pounds of empty body weight for the 229 cows, as compared with 0.65 pound for the bull. Apparently the lung weight of the bull was comparatively low when considered on the basis of the weight of total animal structure.

The weight of the bull's heart was 4.75 pounds or 0.36 pound per 100 pounds empty body weight, as compared to 0.51 pound for the cow and 0.47 pound for 247 of the slaughterhouse cows previously mentioned. When the actual weight of the bull's heart is compared with that of the cow included in this study, or with the group of slaughterhouse cows, it is slightly high, but the weight for each 100 pounds of empty body weight is distinctly low. The measurements of the heart were slightly greater for the bull than for the cow when compared on the basis of the actual units of measurement.

The difference in empty body weight of the cow and the bull was 560.8 pounds as compared with a difference of 573 pounds in the ante-mortem live weight, and although the relation of bull to cow was 161.8 per cent in live weight, it was 173.0 per cent in empty body weight. A comparison of the other post-mortem data of the two animals on the basis of the actual units of weight or measurement shows that the values range from a maximum of 313.0 per cent for weight of thyroid to a minimum of 47.6 per cent for weight of large intestine. Whereas in the ante-mortem data only 3 of the 27 items are below 100 per cent, in the post-mortem data 7 of the 20 items are below 100 per cent.

A good idea of the relative anatomy of the two animals may be had by dividing the 20 items compared on the basis of actual units into seven groups, representing (1) body size, (2) organs of circulation, (3) organs of digestion and assimilation, (4) organs of elimination, (5) endocrine glands, (6) contents of alimentary canal, and (7) visceral fats.

In the body-size group are empty body weight and weight of hide. The average for this group is 191.1 per cent.

The circulation group includes the weight and the two circumferences of the heart. The percentages range from 120.6 to 101.1, and average 111.7 per cent.

The digestion and assimilation group includes the lengths and weights of the intestines and the total weight of empty stomachs. There are seven items in this group with percentages ranging from 124.5 to 47.6 and averaging 99.0 per cent. The three lengths of intestines range from 124.5 to 123.5 and average 124.1 per cent, the three weights of intestines range from 120.4 to 47.6 and average 80.7 per cent, and the total weight of empty stomachs is 78.8 per cent.

The elimination group consists of a single item, the weight of kidneys.

In the endocrine-gland group the percentages range from 313.0 for the weight of thyroid, to 111.9 for the weight of adrenals. The third item in this group, the spleen, has a percentage value of 187.6 and the average for the group is 204.2 per cent. On an average, this group shows a greater difference than any other in favor of the bull. The

two content items are the stomach and the intestines, with an average of 69.8 per cent.

The visceral fats (abdominal and thoracic) average 125.8 per cent.

The percentages in the body-size group would be expected to be similar to those for live weight and for empty body weight. A part of the difference in the weight of the hides of the cow and bull undoubtedly is explained by the fact that the portion of the hide which covered the udder of the cow was not included in the weight. Since the heart is a muscular organ serving all the body tissues, its size might also be expected to be more or less in proportion to the size of the animal. Furthermore, it is not surprising that the percentages for length of intestines approach those for live weight or empty body weight. The lower average percentage for weights of digestive organs may be at least partly due to the fact that the fat was more completely removed from the organs of the bull than from those of the cow. The reason for the low percentage for kidneys and the high average for the endocrine glands is not known. The percentages for contents of digestive tract and for visceral fat probably have comparatively little significance in this study.

When the post-mortem data are compared on the basis of the units per 100 pounds of empty body weight and grouped the same as on the other basis, it is seen that the percentages in the two series are related to each other in the proportion of approximately 173 to 100. The comments made in connection with the first series, therefore, apply also to the second.

SKELETAL STRUCTURE

The method employed in cleaning and mounting the skeleton of the cow was described in a previous publication.⁷ The skeleton of the bull was prepared in the same manner.⁸ The process used in cleaning the bones not only preserved the texture and all the delicate bony structures but prevented injury to the cartilage. The skeletons were cleaned and prepared with very little disarticulation, and mounted according to detailed measurements of the animals made before death.

Different views of the two skeletons and contours of the thoracic cavity and pelvic apertures are shown in Figures 6-12.

Detailed measurements of the two skeletons were made. As far as possible they corresponded to those taken on the living animal, but many additional ones were made. Obviously a measurement of the circumferences of barrel (fore chest, rear chest, and paunch) of the skeletons is impossible because at both the rear chest and paunch planes the ventral wall is missing. Contours, however, can be so drawn as to permit measurement with a greater degree of accuracy, as they represent the exact shape of the available portion of the skeleton at any desired point. Contours, prepared to show the differences in size and shape of the external outlines of the skeletons at the two planes used in measuring the fore chest and the paunch are presented in Figures 13 and 14.

The lower portion of the contours of the paunch can not be completed because the ribs do not meet. Areas of the contours at the

⁷ SWETT, W. W., GRAVES, R. R., and MILLER, F. W. Op. cit.

⁸ The skeleton work was done by the late C. E. Mirguet, an expert osteologist of the Smithsonian Institution, Washington, D. C.

paunch are obtained by connecting the lower extremities of each with a straight line and measuring the inclosed space. These areas, of course, are not comparable to those obtained ante mortem.

In addition to the external skeletal contours at the fore chest and at the paunch, inside contours were made of the anterior aperture of

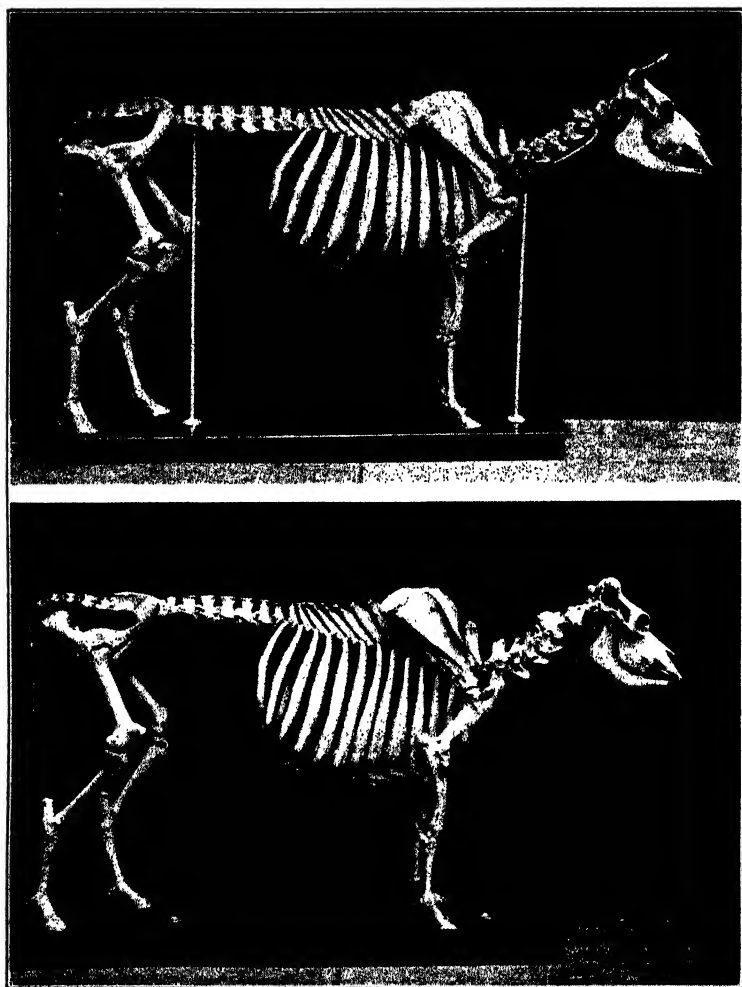


FIGURE 6.—Side view of skeletons (cow, above; bull, below)

the thoracic cage at the first rib, the thoracic cage at the seventh rib and at the thirteenth rib, and the pelvic aperture. The areas of all were measured, but only those for anterior aperture of thoracic cage and pelvic aperture are illustrated. (Figs. 8, 11, and 12.)

The skeletal measurements of the cow and the bull and the relation of the measurements of the bull to those of the cow expressed in

terms of percentage, together with a description of each measurement and the manner in which it was obtained, are presented in Table 4.

TABLE 4.—Detailed skeletal measurements of cow and bull

Item	Cow		Relation of bull's measurements to those of cow
	Centimeters	Centimeters	Per cent
Height at top of highest thoracic spinous process (withers).....	120.00	119.50	99.6
Height at highest point on sacrum.....	121.25	121.25	100.0
Height at top of hip points.....	115.75	116.63	100.8
Height at top of pin bones.....	106.25	114.25	107.5
Length between bearing surfaces:			
Bones of thoracic limb—			
Scapula.....	37.00	39.00	105.4
Humerus.....	27.00	28.50	105.6
Radius.....	28.00	28.00	100.0
Metacarpus.....	20.50	18.50	90.2
Total.....	112.50	114.00	
Average.....	28.13	28.50	* 101.3
Length between bearing surfaces:			
Bones of pelvic limb—			
Femur.....	35.50	38.00	107.0
Tibia.....	33.50	34.00	101.5
Metatarsus.....	23.00	19.75	85.9
Total.....	92.00	91.75	
Average.....	30.67	30.58	* 99.7
Height at poll.....	129.00	122.75	95.2
Length, poll to third thoracic process.....	71.00	70.00	98.6
Length, third thoracic process to point on spine between hips.....	89.50	89.50	100.0
Length, point on spine between hips to rear of pin bones.....	47.00	48.00	102.1
Length, third thoracic process to rear of pin bones.....	136.50	137.50	100.7
Total length, poll to rear of pin bones.....	207.50	207.50	100.0
Sway of back.....	5.00	4.50	90.0
Width of forechest (outside, sixth rib).....	33.25	32.25	97.0
Width of reachest (outside, crossing center tenth rib).....	55.00	53.00	96.4
Width of paunch (outside, crossing center thirteenth rib).....	62.50	64.50	103.2
Depth of forechest (plane of sixth rib).....	68.50	71.00	103.6
Maximum lateral width of thoracic cavity at anterior edge of each rib:			
1.....	9.50	9.75	102.6
2.....	11.50	11.00	95.7
3.....	15.50	14.00	90.3
4.....	19.50	18.00	92.3
5.....	23.50	22.50	95.7
6.....	27.50	26.50	96.4
7.....	33.00	32.50	98.5
8.....	41.50	40.00	96.4
9.....	49.00	47.00	95.9
10.....	55.00	53.00	97.3
11.....	60.00	59.50	99.2
12.....	62.00	62.50	100.8
13.....	62.50	65.00	104.0
Average.....	36.15	35.52	* 98.2
Vertical depth from ventral center of each thoracic vertebra to sternum or to a horizontal line across the lower extremity of the thoracic cage at the eighth to thirteenth vertebra:			
1.....	26.00	22.50	86.5
2.....	31.00	29.00	93.5
3.....	34.00	33.50	98.5
4.....	38.00	36.00	94.7
5.....	41.50	40.00	96.4
6.....	44.50	43.00	96.6
7.....	49.00	45.50	92.9
8.....	47.00	48.50	103.2
9.....	45.00	52.00	115.6
10.....	44.50	49.50	111.2
11.....	45.00	48.50	107.8
12.....	43.00	47.00	109.3
13.....	41.00	45.00	109.8
Average.....	40.73	41.54	* 102.0

* Based on average data of bull and of cow.

TABLE 4.—Detailed skeletal measurements of cow and bull—Continued

Item	Cow	Bull	Relation of bull's measurements to those of cow
	Centimeters	Centimeters	Per cent
Length, center of anterior edge of first rib to junction of thoracic and lumbar vertebrae (length of thoracic cavity).....	76.00	76.50	100.7
Center axes, anterior aperture of thoracic cage:			
Lateral.....	8.00	8.00	100.0
Vertical.....	18.50	22.50	121.6
Contour or cross-section area of anterior aperture of thoracic cage.....	172.90	200.10	115.7
Contour area of thoracic cage (inside) at seventh rib.....	1,365.60	1,701.50	124.8
Contour area of thoracic cage (inside) at thirteenth rib.....	2,135.70	2,315.02	108.4
Contour area (outside) at plane used in measuring fore chest (sixth rib).....	1,439.02	1,421.86	98.8
Contour area (outside) at plane used in measuring paunch (thirteenth rib).....	2,396.23	2,429.48	101.4
Height at anterior dorsal point of sternum (ventral point of anterior aperture of thoracic cage).....	72.00	62.00	86.1
Height at dorsal median posterior point on sternum between attachment of eighth ribs.....	52.50	47.75	91.0
Length of sternum between above points.....	40.00	39.50	98.8
Angle of sternum made with the horizontal.....	(b)	72.5
Length of thoracic cage measured on spine (cervical-thoracic to thoracic-lumbar junctions).....	76.00	77.50	102.0
Average length of each thoracic vertebra (divide above length by 13).....	5.85	5.96	101.9
Length of loin measured on spine (thoracic-lumbar to lumbar-sacral junctions).....	41.00	38.50	93.9
Average length of each lumbar vertebra (divide above length by 6).....	6.83	6.42	94.0
Length of spinous process of each thoracic vertebra:			
1.....	25.00	29.00	116.0
2.....	25.00	29.00	116.0
3.....	24.50	28.50	116.3
4.....	23.50	26.50	112.8
5.....	21.50	23.50	109.3
6.....	20.50	22.00	107.3
7.....	20.00	21.00	105.0
8.....	18.50	20.00	108.1
9.....	17.00	18.00	105.9
10.....	14.50	14.50	100.0
11.....	12.00	12.50	104.2
12.....	9.00	9.50	105.6
13.....	7.50	8.00	106.7
Average.....	18.35	20.15	*109.8
Length of spinous process of each lumbar vertebra:			
1.....	6.50	7.00	107.7
2.....	6.00	7.00	116.7
3.....	5.50	7.00	127.3
4.....	5.50	6.50	118.2
5.....	5.00	6.00	120.0
6.....	4.50	5.50	122.2
Average.....	5.50	6.50	*118.2
Width of each rib, 6 inches from its lowest ossified point (average of right and left):			
1.....	2.13	2.66	124.9
2.....	2.42	2.72	112.4
3.....	3.31	3.59	108.5
4.....	4.42	4.43	100.2
5.....	5.05	5.20	103.0
6.....	5.23	5.51	105.4
7.....	5.81	5.90	101.5
8.....	5.41	4.86	89.8
9.....	4.92	4.34	88.2
10.....	4.69	3.96	84.4
11.....	3.96	3.40	85.9
12.....	3.19	2.86	89.7
13.....	3.29	2.92	88.8
Total.....	53.83	52.35
Average.....	4.14	4.03	*97.3

* Based on average data of bull and of cow.

b Cow, 28° 11'; bull, 21° 9'.

TABLE 4.—Detailed skeletal measurements of cow and bull—Continued

Item	Cow	Bull	Relation of bull's measurements to those of cow
Length of each rib, from vertebral attachment to lowest ossified point (average of right and left):	<i>Centimeters</i>	<i>Centimeters</i>	<i>Per cent</i>
1.....	27.00	30.33	112.3
2.....	32.00	35.00	109.4
3.....	36.00	39.25	109.0
4.....	39.25	43.00	109.6
5.....	43.75	48.00	109.7
6.....	48.00	52.50	109.4
7.....	52.50	56.50	107.6
8.....	55.25	57.88	104.8
9.....	55.25	57.75	104.5
10.....	55.25	57.25	103.6
11.....	54.00	56.00	103.7
12.....	52.25	54.17	103.7
13.....	48.50	49.13	101.3
Average.....	46.08	48.98	*106.3
Width of intercostal spaces (spaces between ribs) approximately 6 inches from lowest ossified point:			
1.....	1.89	2.89	152.9
2.....	2.15	2.91	135.3
3.....	1.06	2.34	119.4
4.....	1.80	2.53	140.6
5.....	1.80	2.09	116.1
6.....	2.76	1.92	69.6
7.....	1.80	1.51	116.2
8.....	3.75	2.44	65.1
9.....	4.51	3.74	82.9
10.....	4.78	4.34	90.8
11.....	4.92	3.49	70.9
12.....	5.07	3.35	66.1
Total.....	36.69	33.55	
Average.....	3.06	2.80	* 91.4
Diameter of foramina of thoracic vertebrae (intervertebral and intra-vertebral):			
1.....	1.22	1.42	116.4
2.....	1.16	1.01	87.1
3.....	1.21	.88	72.7
4.....	1.05	1.11	105.7
5.....	.94	.98	104.3
6.....	.92	.95	103.3
7.....	.94	.95	101.1
8.....	.87	.96	110.3
9.....	.98	.94	95.9
10.....	1.10	.80	72.7
11.....	.84	.89	106.0
12.....	.74	.84	113.5
13.....	2.94	.96	32.7
Average.....	1.15	.98	* 85.1
Diameter of foramina of lumbar vertebrae (intervertebral and intra-vertebral):			
1.....	2.20	1.08	49.1
2.....	2.55	1.82	71.4
3.....	2.79	2.24	80.3
4.....	2.83	2.30	81.3
5.....	2.86	2.92	102.1
6.....	2.24	3.07	137.1
Average.....	2.58	2.24	* 86.8
Length of loin, from center of hip to vertebral attachment of thirteenth rib.....	45.00	43.75	97.2
Width of loin:			
Third lumbar.....	29.00	30.25	104.3
Fourth lumbar.....	30.25	33.25	109.9
Average.....	29.63	31.75	*107.2

* Based on average data of bull and of cow.

* Opening for nerve branch that goes to udder.

TABLE 4.—Detailed skeletal measurements of cow and bull—Continued

Item	Cow	Bull	Relation of bull's measurements to those of cow
	<i>Centimeters</i>	<i>Centimeters</i>	<i>Per cent</i>
Width of hips (outside extremity).....	16.50	47.50	102.2
Width of thurls (outside extremity).....	40.50	41.00	101.2
Width of pin bones (outside extremity).....	27.50	25.50	92.7
Length of rump, top of hip to top of pin bone.....	40.00	39.25	98.1
Angle of rump.....	(^d)	-----	25.4
Angle of floor of pelvis.....	(^e)	-----	102.5
Angle of pelvic aperture.....	(^f)	-----	79.3
Length of pelvic floor.....	17.50	20.50	117.1
Center axes of pelvic aperture:			
Anteroposterior (sacro-pubic).....	25.00	21.00	84.0
Transverse.....	17.20	15.10	87.8
Contour area of pelvic aperture.....	394.30	273.15	71.1
Width between top points of pin bones.....	20.00	14.00	70.0
Width of pelvis immediately above center of acetabulum.....	17.75	13.75	77.5
Width of forehead.....	14.25	18.25	128.1
Width across eyes.....	20.75	24.25	116.9
Length from poll to tip of nose.....	47.00	47.50	101.1
Greatest depth at angle of jaw.....	26.00	25.50	98.1
Measurements of muzzle:			
Outside width of premaxillae (nasal processes)—			
At narrowest point corresponding to corner of mouth.....	7.66	8.58	112.0
Near anterior extremity, corresponding to greatest width of prehensile pad.....	8.28	9.69	117.0
Outside width of mandible (lower jaw)—			
At narrowest point, corresponding to corner of mouth.....	3.50	3.90	114.0
Near anterior extremity, corresponding to lateral limits of incisor teeth.....	7.74	8.48	109.6
Inside diameters of nasal passage—			
Lateral diameter.....	6.72	6.88	102.4
Depth from roof to floor.....	6.05	6.00	99.2
Measurements of bones of thoracic limb (scapula not included):			
Center of shaft of—			
Humerus—			
Circumference.....	14.00	16.50	117.9
Lateral diameter.....	3.92	4.35	111.0
Anterior-posterior diameter.....	4.87	5.85	120.1
Radius—			
Circumference.....	13.00	15.50	119.2
Lateral diameter.....	4.64	5.88	126.7
Anterior-posterior diameter.....	3.17	3.68	116.1
Metacarpus—			
Circumference.....	9.50	10.75	113.2
Lateral diameter.....	3.30	3.95	119.7
Anterior-posterior diameter.....	2.29	2.67	116.6
Average.....	6.52	7.68	* 117.8
Measurements of bones of pelvic limb:			
Center of shaft of—			
Femur—			
Circumference.....	12.50	14.00	112.0
Lateral diameter.....	3.53	4.05	114.7
Anterior-posterior diameter.....	4.30	5.03	117.0
Tibia—			
Circumference.....	11.50	13.25	115.2
Lateral diameter.....	4.10	4.95	120.7
Anterior-posterior diameter.....	2.93	3.57	121.8
Metatarsus—			
Circumference.....	9.50	10.50	110.5
Lateral diameter.....	2.78	3.27	117.6
Anterior-posterior diameter.....	2.90	3.08	106.2
Average.....	6.00	6.86	* 114.2

* Based on average data of bull and of cow.

^d Cow, 13° 44'; bull, 3° 29'.^e Cow, 16° 36'; bull, 17° 1'.^f Cow, 38° 19'; bull, 30° 22'.

TABLE 4.—Detailed skeletal measurements of cow and bull—Continued

Item	Cow	Bull	Relation of bull's measurements to those of cow
Measurements of cervical vertebrae:	<i>Centimeters</i>	<i>Centimeters</i>	<i>Per cent</i>
At greatest width—			
1 (atlas).....	15.30	20.60	134.64
2 (axis).....	10.40	13.70	131.73
3.....	11.10	15.30	137.84
4.....	11.40	16.00	140.35
5.....	11.05	15.20	137.56
6.....	10.90	14.30	131.19
7.....	11.55	14.30	123.81
Average.....	11.67	15.63	* 133.90
At greatest depth—			
1 (atlas).....	9.68	10.08	104.13
2 (axis).....	13.73	15.33	111.65
3.....	11.70	14.23	121.62
4.....	12.80	14.60	114.06
5.....	13.88	17.15	123.56
6.....	18.20	20.65	113.46
7.....	20.23	24.60	121.60
Average.....	14.32	16.66	* 116.38

* Based on average data of bull and of cow.

The skeletons of the cow and bull were almost the same in height. The percentages of 100.8 for height at hips and 107.5 for height at pin bones signify that the slope of rump was less for the bull than for

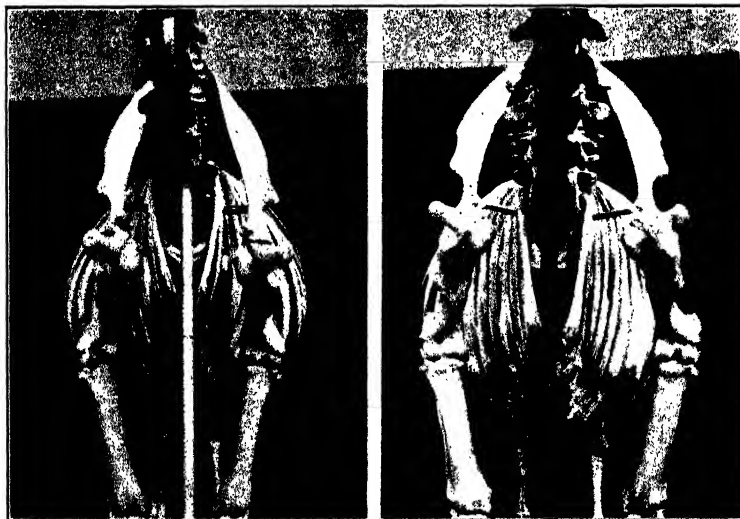


FIGURE 7.—Front view of skeletons (cow, left; bull, right)

the cow. In view of the similarity in height of body, one would expect the length of the bones of the thoracic and pelvic limbs of the bull to be similar to those of the cow, and Table 4 indicates that such

is the case. The total length of the four bones of the thoracic limb is 101.3 per cent as great for the bull as for the cow. A similar relationship is found in the length of the bones of the pelvic limb, the percentage being 99.7. The total length of the fore and the rear legs of the bull is almost identical with that of the cow, but the upper units are distinctly longer and the lower units distinctly shorter in the bull. The advantage of this is that it gives the bull additional strength for defense and for carrying his weight during copulation. The height at poll is 6.25 cm less for the bull than for the cow, but since this is determined almost entirely by the angle at which the thoracic and cervical portions of the spinal column are joined in assembling the skeleton, little significance is attached to this difference.

The length from poll to third thoracic process (withers) is probably the least significant measurement in this group because of the extent to which it is affected by the height and position of the head and neck.

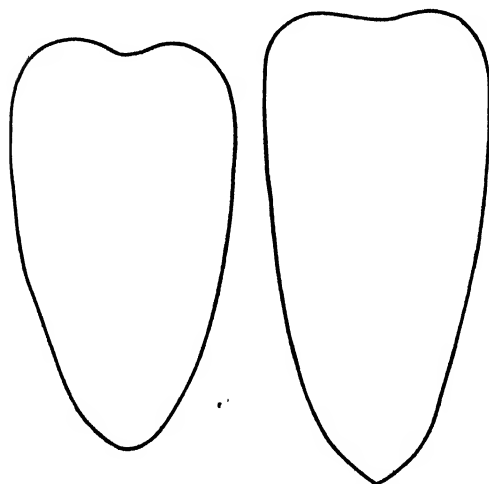


FIGURE 8.—Contours of anterior aperture of thoracic cavity (cow, left; bull, right)

However, the average of the five percentages representing general body lengths is 100.3, which shows that the measurements of body length are even more similar for the two skeletons than the measurements of height.

For two animals varying so greatly in size there is surprising similarity in the widths of the fore chest, rear chest, and paunch. Not one of these measurements differs by more than 2 cm (less than 1 inch), and the relation of width of bull to width of cow at these three points averages 98.9 per cent. The maximum lateral width of the

thoracic cavity taken at the anterior edge of each rib is less for the bull at 10 of the 13 ribs, but on an average the two animals are strikingly similar.

At the fifth, sixth, and seventh ribs, which represent the region of the plane used in measuring the fore chest, the percentages average 96.9; at the tenth rib, the plane of the rear chest, the percentage is 97.3; and at the thirteenth rib, the plane at which the paunch measurements were taken, the percentage is 104.0. The lowest percentage is for the third rib and the highest percentage is for the thirteenth. Ante-mortem measurements of the chest are not possible at the plane represented by the lowest percentage because of the position of the scapula and humerus. The generally progressive increase in percentage from the third to the thirteenth rib and the ratio of the widths of paunch to chest show definitely that the lateral wedge of the skeleton was greater for the bull than for the cow, whereas the ante-mortem wedge shape was less.

The outside depth of fore chest at the sixth rib was 103.6 per cent as great for the bull as for the cow. Obviously a depth at the tenth and thirteenth ribs which would be comparable to the ante-mortem measure-



FIGURE 9.—Rear view of skeletons (cow, left; bull, right)

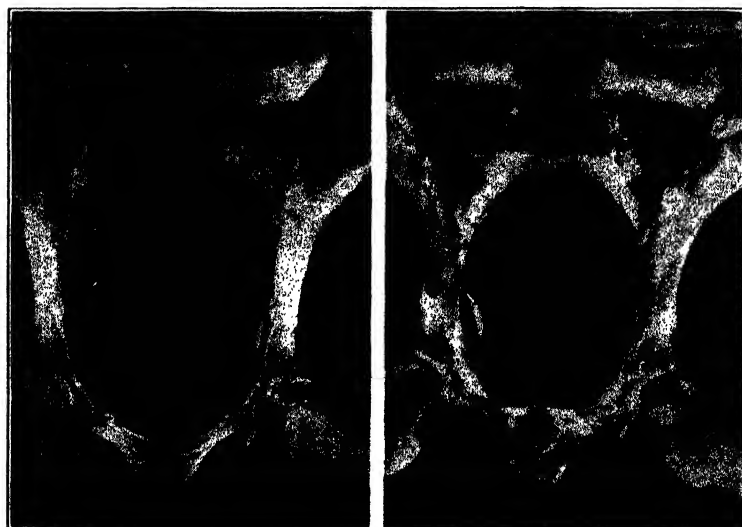


FIGURE 10.—View of anterior pelvic aperture in each skeleton, taken to the same scale from below and in front, with camera approximately perpendicular to the rim of the pelvis (cow, left; bull, right)

ment at the same plane, could not be obtained. The two skeletons are, however, compared in depth by measuring, in a vertical plane, the depth from the ventral center of each thoracic vertebra to the dorsal

surface of the sternum at each vertebra from the first to the seventh, inclusive, and to a horizontal line across the points forming the lower extremity of the thoracic cage at each vertebra from the eighth to the thirteenth, inclusive. At each of the first seven vertebrae the bull is shallower than the cow. For the next six vertebrae the depth is in each case greater for the bull. On an average, the depths are similar for the two skeletons. The fact that the bull is 3.5 cm shallower than the cow (86.5 per cent) at the first thoracic vertebra and 4.0 cm deeper (109.8 per cent) at the thirteenth thoracic vertebra, together with the fact that all depths from the first to the seventh vertebrae are less for the bull (average 94.2 per cent), whereas those from the eighth to the thirteenth vertebrae are all greater (average 109.5 per cent), shows a decidedly greater vertical wedge for the bull than for the cow. Although the ante-mortem and post-mortem measurements for depth of body are not comparable because much of the

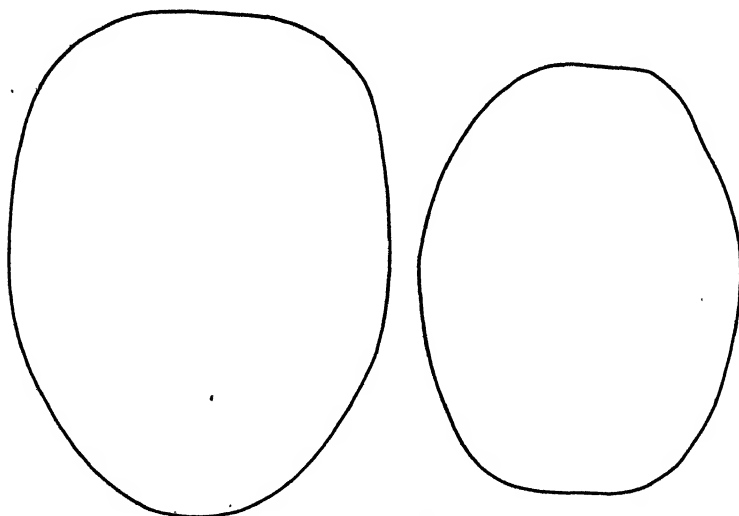


FIGURE 11.—Contours of the anterior pelvic aperture (cow, left; bull, right)

ante-mortem depth in the abdominal region is nonskeletal, the bull in each instance appears to have a greater vertical wedge shape than the cow. (Fig. 6.)

In length of thoracic cavity the two skeletons are almost the same whether measured from center of anterior edge of first rib to thoracic-lumbar junction, or from cervical-thoracic to thoracic-lumbar junctions. The lateral axis of the anterior aperture of the thoracic cage is exactly the same in both skeletons, but the vertical axis is 4.0 cm greater for the bull. Contour areas of the inside of the thoracic cage at the anterior aperture (fig. 8), at the plane of the seventh rib and at the plane of the thirteenth rib, average 116.3 per cent as great for the bull as for the cow. The outside contour areas at the sixth and thirteenth ribs, the planes used in obtaining the ante-mortem measurements of the fore chest and paunch, are, respectively, 98.8 and 101.4 per cent as great for the bull as for the cow. (Figs. 13 and 14.)

The sternum of the bull is much lower in front, considerably lower in the rear, of almost the same length, and is set at an angle of $21^{\circ} 9'$ as compared with $29^{\circ} 11'$ for the cow. (Fig. 6.)

The bull is considerably shorter than the cow in the region of the loin. Although the thoracic vertebrae average 0.11 cm longer, the lumbar vertebrae average 0.41 cm shorter for the bull than for the cow. The fact that the lumbar portion of the vertebral column is actually 2.5 cm shorter in a bull than in a cow weighing 573 pounds less is worthy of note. The additional length of loin in the cow may serve the purpose of providing more room for the development and delivery of the fetus. The shorter loin in the bull may give him added strength for his activities in fighting and in copulating.

In every instance the thoracic spinous processes are of equal or greater length in the bull, averaging 9.8 per cent longer than in the cow. The greatest difference is in the spinous processes attached to the anterior vertebrae, the first 6 of which average 113.0; whereas the 6 posterior ones average 105.1, as compared to 108.7 per cent for the entire 13. (Figs. 6 and 15.) Every one of the lumbar spinous processes also is longer, averaging 1.0 cm, or 18.2 per cent greater in the bull. (Figs. 6 and 16.)

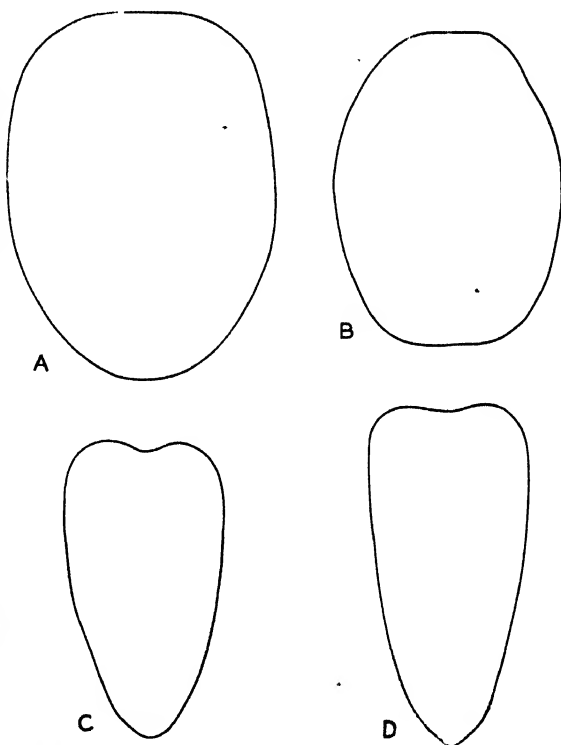


FIGURE 12.—Contours of anterior pelvic aperture and anterior aperture of thoracic cavity, shown together to illustrate relative size of each; A, Anterior pelvic aperture of cow; B, anterior pelvic aperture of bull; C, anterior aperture of thoracic cavity of cow; D, anterior aperture of thoracic cavity of bull

The ribs differ considerably in width, length, and position. (Fig. 6.) They average 0.11 cm less in width for the bull. The anterior ribs of the bull are wider, but the posterior ribs are narrower than those of the cow, the percentages for width averaging 109.1 for the 6 anterior ribs, but only 87.8 for the 6 posterior ribs, whereas the entire 13 average 98.7. The bull's ribs average 2.9 cm longer than those of the cow. The greatest differences in length also are found

in the anterior ribs, the first 6 averaging 109.9 per cent, but the 6 posterior ribs only 103.6 per cent. There are, of course, only 12 intercostal spaces on each side, and they average 0.26 cm narrower in the bull. In length of thoracic spinous processes, width of ribs, and length of ribs the relation of bull to cow is markedly different in the anterior portion of the thoracic cavity from what it is in the posterior. In width of intercostal spaces this difference is even more striking, the percentages averaging 122.3 for the 6 anterior spaces,

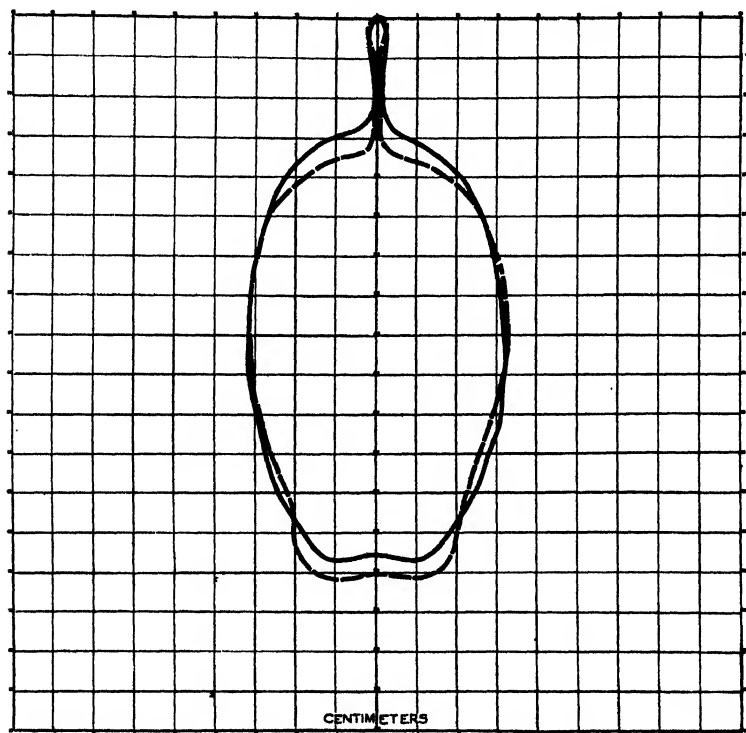


FIGURE 13.—External contours of fore chest of skeletons (cow, solid line; bull, broken line)

but only 82.0 for the 6 posterior spaces, as compared to 102.2 for the 12 spaces.

The significance of openness of conformation, as an indication of size of intervertebral and intravertebral foramina and of the freedom of passage of nerves from the spinal cord, was discussed in a previous publication.⁹ It was pointed out that intravertebral foramina are more numerous than intervertebral foramina, and consequently that the width of spaces between vertebrae would not be expected to be of great importance as an indication of freedom of passage of nerves. There is some indication that intervertebral foramina are relatively more numerous in the lumbar than in the thoracic portions. The method of measuring the foramina was the same as that outlined in

⁹ SWETT, W. W., GRAVES, R. R., and MILLER, F. W. Op. cit.

the previous publication. Some of the foramina were elongated and somewhat irregular in shape, but the measurement recorded as the diameter is the greatest transverse dimension measurable. Some vertebrae had both intervertebral and intravertebral foramina. Some were divided by cartilage or bone into two parts. Whenever a vertebra had two foramina or one divided into two parts, both were measured and the total of the two diameters was recorded. (Figs. 15 and 16.)

The thoracic foramina average 0.17 cm less in diameter, or only 85.1 per cent as much for the bull. In eight of the cases recorded

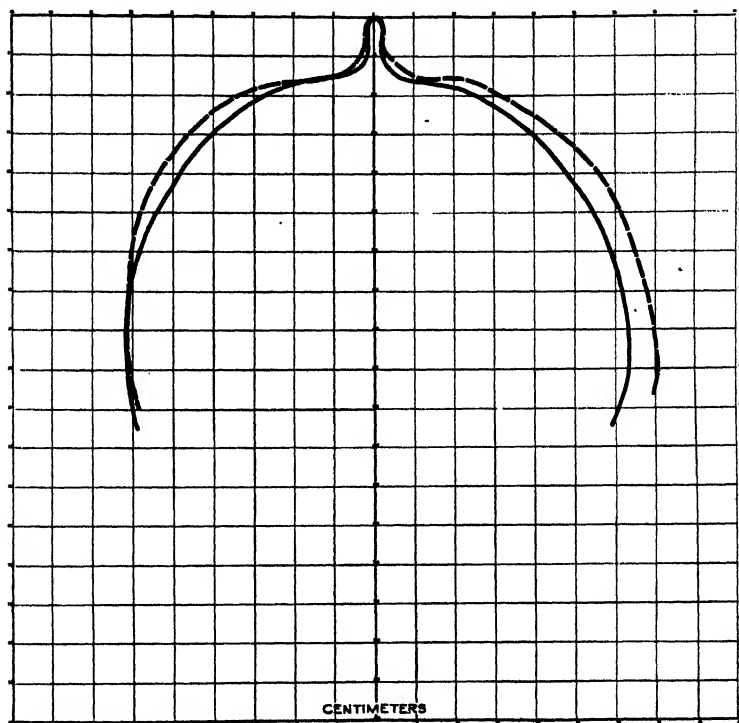


FIGURE 14.—External contours of paunch of skeletons (cow, solid line; bull, broken line)

the measurement was greater for the bull, and in five it was less. The average of the percentages for the 6 anterior vertebrae is 98.3; for the 6 posterior ones it is 88.5; and for the entire group of 13 it is 94.0. On an average the lumbar foramina are 0.34 cm less in diameter, or 86.8 per cent as large in the bull.

Each of the percentages for lumbar foramina is greater than that for the next anterior vertebra. The reason for this steady increase in percentages is not understood. It was pointed out in comparing the dairy cow with the beef cow, that the nerves which lead to the udder leave the spinal cord and pass through foramina between the second and third lumbar vertebrae. The foramen at this location is only 71.4 per cent as large in the bull—a difference in diameter of 0.73 cm. The significance of size of foramina appears to be still in doubt.

The length of loin, measured from the center of the face of the hip to the attachment of the thirteenth rib, is less for the bull, but the width of loin as measured at the third and fourth lumbar vertebrae is considerably greater. The widths of hips, thurls, and pin bones are, respectively, 1.0 cm greater, 0.5 cm greater, and 2.0 cm less for the bull.

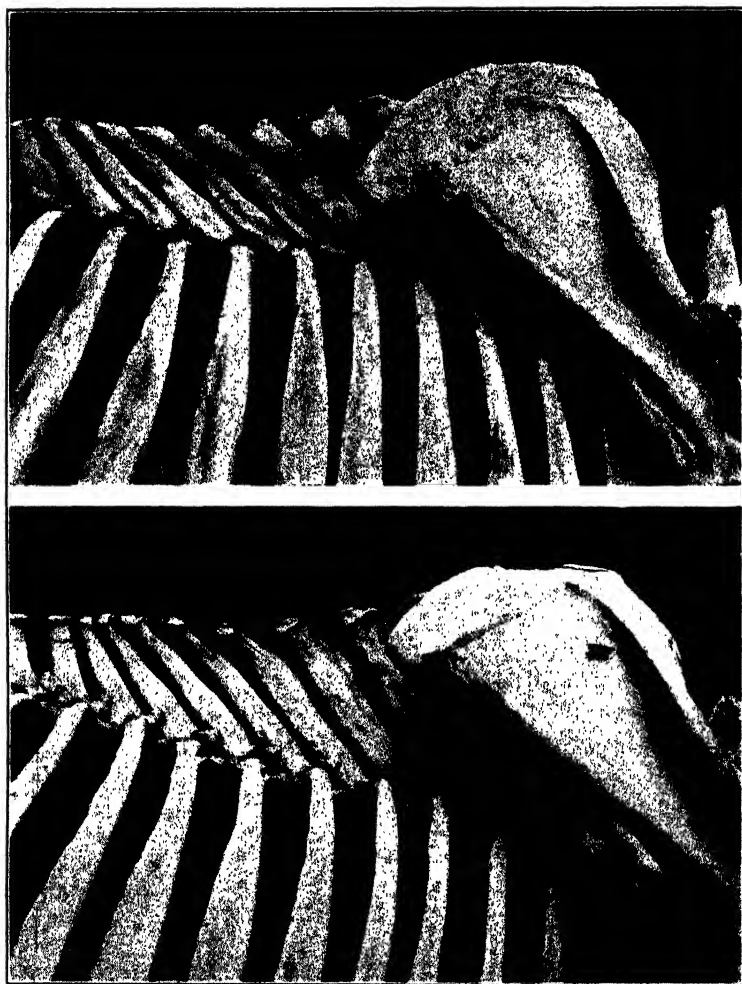


FIGURE 15.—Thoracic vertebrae, ribs, and spinous processes, showing foramina (cow, above; bull, below)

This difference in width at pin bones, which is rather high for so small a measurement, is an indication of the narrowness of the pelvis in the male as compared with that of the female. The length of rump of the two skeletons is almost the same. The angle or slope of rump is greater for the cow, the angle of the pelvic floor is almost the same for

both skeletons, and the angle of pelvic aperture is greater for the cow. Although the skeletons were assembled according to the measurements taken on the living animals, the possibility that any of these angles might have been affected by the final assembly is recognized. The

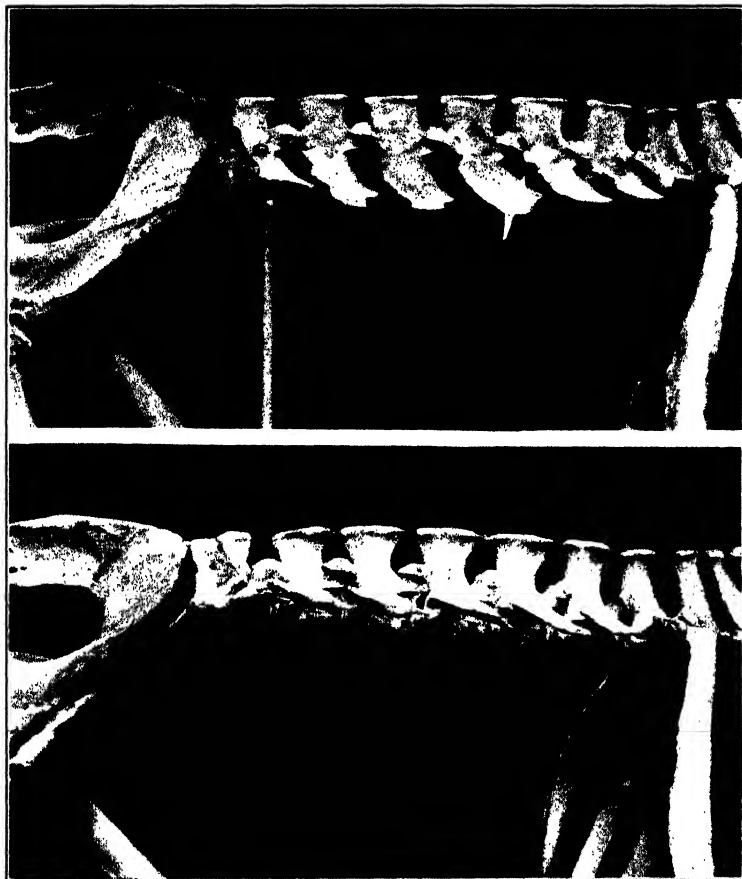


FIGURE 10.—Lumbar vertebrae and spinous processes showing foramina (cow, above; bull, below). Arrows indicate foramina that permit passage of nerve which supposedly innervates the mammary gland

length of pelvic floor is 3.0 cm greater in the bull, a difference of 17.1 per cent, which is relatively high.

The difference in size and shape of pelvis is one of the most striking found in this study. (Figs. 10 and 11.) In the previous publication¹⁰ the data for lateral and longitudinal axes of the anterior pelvic aperture were interchanged through error. In this discussion the error is corrected and the terms "anteroposterior (sacro-pubic)" and "transverse" are used to indicate the axes measured. Both are much smaller in the bull. The contour of the anterior pelvic aperture of each

¹⁰ SWETT, W. W., GRAVES, R. R., and MILLER, F. W. Op. cit.

skeleton was drawn and its area measured. As a result of the redrawing, the area of the cow is slightly different from that previously published. In this comparison the area of the anterior pelvic aperture of the bull is only 71.1 per cent as great as that of the cow. The area of the anterior pelvic aperture of the bull is only 66.3 per cent of what it would be if it were in proportion to the ante-mortem width of hips, thurls, pin bones, and loin; only 69.6 per cent as great as it would be if it were in proportion to the skeletal width of hips, thurls, pin bones, and loin; and only 41.1 per cent of what it would be if it were in proportion to the total animal structure or empty body weights of the two animals. This is illustrated in another way by Figure 12, which shows the contours of the anterior apertures of the pelvis and of the thoracic cavity of each skeleton. The area of the anterior pelvic aperture of the bull is only 61.4 per cent as great as it would be if it

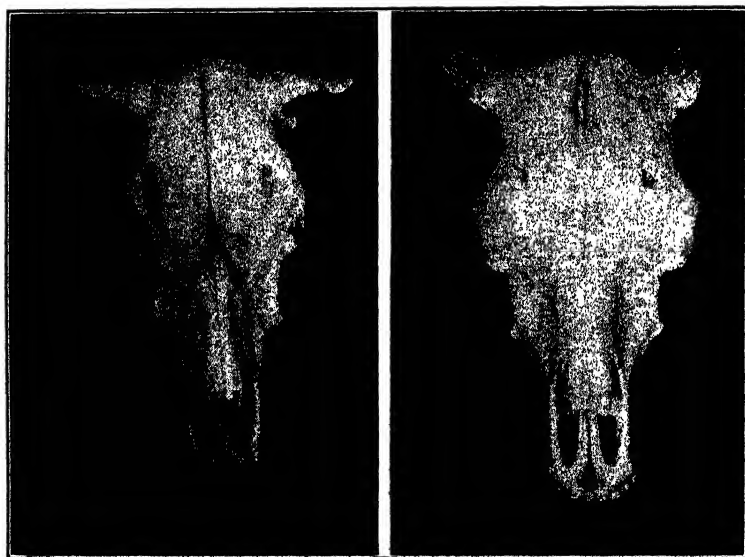


FIGURE 17.—Front view of the skulls (cow, left; bull, right)

were in proportion to the areas of the anterior aperture of the thoracic cavity. The posterior aperture also—in fact the entire pelvic cavity—is distinctly less roomy in the bull. The width between the top of the pin bones and the width above the acetabulum are both very much less in the bull. The greater roominess of the pelvic cavity in the female is one of the most striking differences between the two animals and nicely illustrates the provision of nature for the delivery of the fetus.

In a group of 10 measurements of the head, 8 are larger for the bull. The two that are smaller for the bull are greatest depth at angle of jaw and depth of nasal passage, but the percentages for these measurements indicate that the differences are of little significance. The greatest individual differences in head measurements are, in the order named, width of forehead, outside width of nasal processes, and width across eyes. (Fig. 17.) The length from poll to tip of nose

(the only head length measured) is nearly the same for the two animals (101.1 per cent); the widths of head average 114.3; and the depths of head average 98.7 per cent. The greatest differences in head measurements are, therefore, in width.

Figure 6 shows little difference in the heads as viewed from the side, but indicates a marked difference in heaviness of cervical vertebrae. The cervical vertebrae of the bull are greater, averaging 3.96 cm more in width and 2.34 cm more in depth. The outstanding difference between the measurements of the head of the cow and of the bull appears to be the difference in width.

The circumference and the lateral and anterior-posterior diameters of the bones of the thoracic limb (except the scapula, which was not measured), and of the pelvic limb, are in every instance greater for the bull.

INTERRELATIONS OF ANTE-MORTEM, POST-MORTEM, AND SKELETAL-STRUCTURE DATA

The ante-mortem and skeletal-structure data which appear to be representative of body size, or which might be considered as having an association with the functions of circulation and respiration or of digestion and assimilation, have been grouped in a manner similar to that followed in discussing the post-mortem data. The percentages showing the relation of the bull's measurements to those of the cow, for the ante-mortem, post-mortem, and skeletal-structure data, are assembled in Table 5, according to the functional grouping outlined.

In the data for body size the seven ante-mortem items are much greater for the bull. The first (live weight) and the last two (volume of barrel and body-surface area) are largely dependent on the muscular development and fleshing of the animal. They average 144.3 per cent. The other four, which are more significant of body dimensions, average only 103.7 per cent. The two post-mortem items average 191.1 per cent, but since empty body weight is almost entirely dependent, and weight of hide is to some extent dependent, on fleshing, such a high percentage is not surprising. The four skeletal measurements average 100.9 per cent. It is apparent, therefore, that the differences in the body-size group are due almost entirely to the degree of fleshing of the animal.

In the circulation and respiration group also, the ante-mortem items, which are measurements of the fore chest, are much greater for the bull. In this group also, the width, circumference, and especially the contour-area measurements, are affected materially by the fleshing of the animal. As the lung weights were not compared, the three post-mortem items refer to circulation but not to respiration. They are somewhat greater for the bull. The four skeletal-structure items are only slightly greater for the bull, indicating again the effect of fleshing on the ante-mortem differences.

In the digestion and assimilation group also the ante-mortem items are much greater for the bull. The seven post-mortem items average 99.0 per cent, and the three skeletal-structure items average 104.3 per cent.

The average percentages showing the relation of bull to cow indicate that the bull's organs of circulation were relatively large in proportion to the skeletal development in the fore chest, while the organs of digestion and assimilation were relatively small in proportion to the skeletal measurements of the paunch.

TABLE 5.—*Ante-mortem, post-mortem, and skeletal-structure data grouped on the basis of their association with body size, circulation and respiration, and digestion and assimilation*

Type of data	Data for body size		Data for circulation and respiration		Data for digestion and assimilation	
	Item	Relation of bull's measurements to those of cow	Item	Relation of bull's measurements to those of cow	Item	Relation of bull's measurements to those of cow
Ante-mortem...		Per cent		Per cent		Per cent
	Live weight.....	161.8	Depth, fore chest.....	102.8	Depth, paunch.....	112.4
	Height at withers.....	99.9	Width, fore chest.....	138.2	Width, paunch.....	122.2
	Width of hips.....	104.7	Circumference, fore chest.....	117.6	Circumference, paunch.....	116.5
	Length, withers to pin bones.....	101.1	Contour area, fore chest.....	146.8	Contour area, paunch.....	141.3
	Length, poll to mouth (tape).....	108.9				
	Volume of barrel.....	149.0				
	Body surface area.....	122.0				
	Average.....	121.1		126.4		123.1
Post-mortem...	Empty body weight.....	173.0	Weight of heart.....	120.6	Weight, small intestine.....	120.4
	Weight of hide.....	209.1	Circumference of heart (base).....	101.1	Weight, large intestine.....	47.6
			Circumference of heart (apex).....	113.5	Total weight, intestines.....	74.2
					Length, small intestine.....	124.5
					Length, large intestine.....	123.5
					Total length, intestines.....	124.3
					Total weight, stomachs.....	78.8
	Average.....	191.1		111.7		99.0
Skeletal structure.	Height at withers.....	99.6	Depth, fore chest.....	103.6	Width, paunch.....	103.2
	Width of hips.....	102.2	Width, fore chest.....	97.0	Contour area, paunch (inside thoracic cage at thirteenth rib).....	103.4
	Length, withers to pin bones.....	100.7	Contour area chest (inside, seventh rib).....	124.8	Contour area, paunch (outside thirteenth rib).....	101.4
	Length poll to tip of nose (caliper).....	101.1	Contour area fore chest (outside sixth rib).....	98.8		
	Average.....	100.9		106.1		104.3

The ante-mortem averages for the three groups (121.1, 126.4, and 123.1 per cent) average 123.0 per cent, and are very similar; the post-mortem averages, aside from those of the body-size group, which is dependent to a very high degree on condition and fleshing, are considerably lower (111.7 and 99.0 per cent); and the skeletal-structure items also are lower (100.9, 106.1, and 104.3 per cent). Considered on any basis, the data emphasize the similarity of the two animals from the standpoint of body dimensions, internal anatomy, and skeletal structure, and show that the differences which existed were to a very great extent due to differences in the muscular development and fleshing of the animals.

SUMMARY

EXTERNAL CONFORMATION OF LIVING ANIMAL

Although the bull was 61.8 per cent heavier than the cow, his body lengths and heights were, on an average, not more than 2 per cent greater.

The bull's depths averaged 7.4 per cent, and his widths and circumferences averaged, respectively, 17.5 and 17.8 per cent greater.

On an average, the depths, widths, and circumferences of barrel (fore chest, rear chest, and paunch measurements) were 7.4, 26.4, and 15.5 per cent greater for the bull.

In the entire group of 25 conformation measurements, only 3 were less for the bull, and the average was 10.7 per cent greater for the bull.

The averages for each group of conformation measurements show that the bull was larger, but the differences in measurements do not approach the difference in live weight.

The bull was 46.8 per cent greater in contour area of fore chest and 41.3 per cent greater in contour area of paunch. The proportion of the total area above the vertical mid-point of the contours of the fore chest and of the paunch were almost identical for the two animals. The distribution of the area of the paunch indicated for both animals a deep but "slab-sided" body.

The cow and bull were similar in respect to slope of rump.

The thoracic and abdominal indexes indicate that the cow was deep and narrow and the bull relatively wide in the fore chest but that both were similar at the paunch.

The bull was 49.0 per cent larger in volume of barrel and 22.0 per cent larger in body-surface area.

In legginess very little difference between the two animals was found.

The wedge shape of the bull was greater than that of the cow from the standpoint of depth, but from the standpoint of width, circumference, and contour area it was less.

The circumference of shin bone or metacarpus as measured in the living animal indicated considerably greater heaviness of bone in the bull.

The greatest conformation differences were found in widths and circumferences. The nine conformation measurements showing the greatest difference between cow and bull were all for width or circumference. Only moderate differences were noted between the depths of body of the two animals. The smallest differences were in heights and lengths, which indicates that the relative "scale" of the two animals was almost the same.

INTERNAL ANATOMY

The empty body weight of the bull was 560.8 pounds, or 73.0 per cent greater than that of the cow. The corresponding ante-mortem differences of 573 pounds and 61.8 per cent are similar.

The total lengths of intestines for the cow and bull were, respectively, 166.50 and 206.96 feet. Although this difference is considerable, the length recorded for the cow is at least 30 feet above the minimum, and that for the bull is about 35 feet below the maximum, recorded in a post-mortem study of a large number of animals, now in progress.

Retention of blood in the lungs of the cow prevented the determination of her lung weight. That of the bull was 8.70 pounds, which on

the basis of data obtained from a large number of cows, indicates that the weight of lungs for each 100 pounds of empty body weight was decidedly low.

In weight and in circumference over the apex, the heart of the bull was considerably greater than that of the cow. In circumference near the base the hearts were almost the same. When compared with the weight of heart of the cow used in this study, or with the average for a large number of cows on which post-mortem data are available, the weight of heart of the bull for each 100 pounds of empty body weight is low.

The 20 post-mortem weights and measurements compared were 125.1 per cent as great for the bull as for the cow. Thirteen of these were above 100 per cent and averaged 154.2 per cent. Of the 7 items below 100 per cent, only the 2 which represented total weight of empty stomachs and weight of kidneys were comparable or significant; the 2 items which represented contents of digestive tract are affected by conditions of management; weight of thoracic fat is probably of little significance; and the other two items, which represented weight of intestines, might have been affected by the writers' inability to remove all the fat. The average of the 13 items above 100 per cent and the 2 below 100 per cent that may be significant, was 144.8 per cent.

The relation of bull to cow appears to have been 191.1 per cent for the post-mortem items directly associated with body size, 111.7 per cent for those pertaining to circulation, 99.0 per cent for the organs of digestion and assimilation, 87.8 per cent for the kidneys, 204.2 per cent for the endocrine glands studied, and 125.8 per cent for the visceral fats.

Except for the weights of kidneys, total stomachs, and total intestines, the weights and measurements of the organs and body parts were rather consistently greater for the bull.

From the standpoint of units of weight or measurement for each 100 pounds of empty body weight, only four items were greater for the bull. These are weight of thyroid, weight of hide, which is directly associated with body size, weight of spleen, and weight of abdominal fat, which probably is of little significance. The average for the 19 items is 70.9 per cent. The average for the four items above 100 is 129.0 per cent. It appears, therefore, that aside from some of the endocrine glands, the difference in size of organs was not in proportion to the difference in the empty body weight of the two animals.

The only organs found to be proportionately larger in the bull were the thyroid and the spleen.

SKELETAL STRUCTURE

The skeletal heights were almost the same for the bull as for the cow. This should have been the case since the skeletons were assembled on the basis of ante-mortem height measurements, which were nearly the same for both animals.

The total length of the leg bones was almost the same for both animals. In both the thoracic and pelvic limbs, however, the upper (dorsal) bones were longer in the bull, and the lower (ventral) bones were longer in the cow.

The skeletal body lengths of the two animals were almost identical.

Outside widths, taken as nearly as possible at the planes used in measuring the ante-mortem widths of fore chest, rear chest, and

paunch, were strikingly similar for the two animals. In no instance was the difference greater than 2 cm.

The widths of thoracic cavity taken at the anterior edge of each rib were similar, but averaged slightly less for the bull than for the cow. They do, however, indicate a somewhat greater lateral wedge for the bull, which is the reverse of the relation shown by the ante-mortem measurements.

On an average, the inside depths of the thoracic cavity of the bull differed from those of the cow by less than 1 cm. They were, however, less in front and greater in the rear, showing a considerably greater vertical wedge for the bull. This is in agreement with the ante-mortem data.

The length of thoracic cavity and the average length of thoracic vertebrae were almost the same for the two animals.

Although the average widths, average depths, and the length of the thoracic cavity of the cow and bull were very similar, the inside cross section or contour areas measured were distinctly greater for the bull. The outside contour areas at the fore chest and at the paunch, however, were almost the same for both animals.

The sternum of the bull was much lower in front, considerably lower in the rear, almost the same in length, and slightly more nearly level than the sternum of the cow.

The loin was considerably shorter and wider in the bull.

The dorsal spinous processes, both thoracic and lumbar, were distinctly longer in the bull, but very little difference in their position was noted. The greatest differences in thoracic processes were found in those attached to the anterior vertebrae and may have had some relation to the "crest" in the bull.

The lateral processes attached to the lumbar vertebrae were nearly horizontal in the bull, whereas those in the cow inclined downward.

The anterior ribs of the bull were wider and the posterior ribs were narrower, but on an average they were nearly the same for both animals.

The length of every one of the ribs was greater for the bull, but the largest relative differences were found in connection with the anterior ribs.

The average width of the spaces between ribs was slightly greater in the bull; but the anterior ones were very much greater, and the posterior ones very much less in the bull.

Although there was a great deal of variation in individual vertebrae, both the thoracic and lumbar foramina were on an average distinctly smaller in the bull. The one that is supposed to permit passage of the nerve which in the cow innervates the udder, was only 71.4 per cent as large in the bull as in the cow. The significance of the size of foramina appears to be speculative.

The pelvis of the bull was strikingly different from that of the cow in many respects, although the width of hips and thurls and the length of rump of the two animals were nearly the same. The slope of the rump was considerably less in the bull, the angle of the pelvic floor was about the same in both animals, and the angle of the anterior pelvic aperture was somewhat less in the bull. The floor of the pelvis was longer in the bull. The axes of the anterior pelvic aperture were both distinctly less in the bull, and its contour area was only 71.1 per cent as great in the bull as in the cow. The width between the top points of the pin bones, and the width of pelvis above the acetabulum were both

very much less in the bull. The entire pelvic cavity was distinctly less roomy in the bull.

The width of head was distinctly greater in the bull, but the other head measurements differed only slightly.

The cervical vertebrae were noticeably larger in the bull, particularly in width.

The similarity in measurements of height and of length of the entire skeleton, as well as its parts, indicates that both skeletons were almost the same in "scale."

The circumference and the lateral and anterior-posterior diameters of the leg bones, except the scapula, which was not measured, were in every instance greater in the bull. A relatively greater heaviness of bone in the bull was indicated.

A comparison of large numbers of cow and bull skeletons would be desirable but impracticable. The data obtained in this study are presented to show the differences found between two skeletons, and it is not to be inferred that the same differences would necessarily be found between skeletons of all cows and bulls.

CONCLUSIONS

The external conformation of the bull differed considerably from that of the cow. The most marked differences noted were in widths and circumferences of the body and in general heaviness in the anterior parts. In body depths the animals were only moderately different, and in the measurements which indicate "scale" they were almost the same.

The apparent differences in the conformation of the cow and bull are due largely to (1) the "crest" of the bull, which is partly skeletal but mostly muscular; (2) the low front chest development in the bull, which gets its appearance partly from the heavy muscular development, and partly from the low position of the anterior end of the sternum bone, but largely from the abundance of loose skin that extends from the throat to the brisket; and (3) the fact that the head of the bull, although similar in length and depth to that of the cow, is much broader. Any tendency for the bull to appear light in the flank is due in part to his relatively heavy muscular development in the front quarters, and to the fact that he does not, like the cow, have an udder to carry out his underline on a downward slope.

Although many of the bull's internal organs were actually larger than those of the cow in units of weight or measurement, only two of them were proportionately as large as in the cow when considered on the basis of total animal structure or empty body weight. The differences in size of internal organs do not appear to be sufficiently great to indicate significant differences in functional ability.

In skeletal structure the cow and bull were very similar. The greatest differences appear in the cervical vertebrae and in the size and shape of the pelvis. The first gives the bull the necessary strength to defend himself; the second is undoubtedly a provision of nature to enable the cow to give birth to her young.

The cow and bull were surprisingly similar in some of their body dimensions, and in their internal anatomy and skeletal structure. The differences which did exist were to a very great extent due to differences in muscular development and fleshing.

EFFICIENCY FACTORS AND THEIR USE IN DETERMINING OPTIMUM FERTILIZER RATIOS¹

By W. A. HUELSEN

Associate Chief in Olericulture, Department of Horticulture, Illinois Agricultural Experiment Station

INTRODUCTION

The conventional methods of interpreting the results of field experiments with fertilizers may not prove to be entirely satisfactory under all circumstances. For example, in a single experiment involving a large number of fertilizer ratios, a situation may arise wherein yields having no statistically significant differences are derived from several treatments which appear to have only a remote resemblance to each other. Under such conditions it is virtually impossible to determine the optimum fertilizer ratio, and the investigator is compelled either to guess or to have recourse to supplementary experiments of various types. Economic interpretations based on fertilizer costs are possible, of course, and are frequently used, but it is doubtful whether their use is justified in experiments in which the primary object is to determine the optimum ratios.

The experiment discussed in this paper, because of the large number of treatments involved, proved to be extremely difficult to interpret. Of the 63 fertilizer treatments included in the work, the 10 highest ranking ratios proved to be not only quite diverse, but also to have a maximum difference in increased yields over adjacent checks of only 0.323 ± 0.162 ton, or 20.85 ± 9.4 per cent. (Table 1.) Neither one of these differences is statistically significant, and yet these 10 treatments are quite dissimilar as to ratio. In this case supplementary experiments were conducted in several sections of Illinois on many different types of soils, with the result that the optimum ratios were found to be included among the 10 mentioned.

Conducted in this way, fertilizer experiments become unduly burdensome and expensive, because of the large number of treatments which must be included in the supplementary work. The question naturally arises, Is it not possible to submit the results of the original experiment to a more intensive and critical analysis than is usually given them? Would such an analysis narrow the range of treatments to be considered and lead to the determination of the optimum ratio without recourse to supplementary tests? The purpose of this article is to outline a method by which this may be done and to illustrate it by analyzing an extensive fertilizer experiment.

¹ Received for publication Nov. 9, 1931; issued November, 1932.

TABLE 1.—Mean increases in sweet-corn yields per acre in fertilized plots over respective adjacent checks

Treatment	Edible ears				Green fodder			
	Tons increase over check	Dev. P. E.	Student's odds	Percentage increase over check	Tons increase over check	Dev. P. E.	Student's odds	Percentage increase over check
001	-0.193±0.058	3.3	33:1	-8.98±2.7	-0.452±0.265	1.7	6:1	-12.24±7.2
002	.030±.034	0.9	2:1	1.63±1.6	.162±.108	1.5	5:1	5.07±3.4
004	.066±.058	1.1	3:1	3.58±3.2	-.078±.109	.7	2:1	-2.44±3.5
010	.397±.084	4.7	179:1	22.63±4.8	.432±.110	3.9	73:1	15.64±4.0
011	.244±.070	3.5	43:1	13.91±4.0	.303±.104	2.9	22:1	10.97±3.8
012	.338±.095	3.6	46:1	17.99±5.0	.410±.131	3.1	28:1	13.58±4.4
014	.519±.102	5.1	276:1	27.62±5.4	1.188±.152	7.8	4,999:1	39.36±5.0
020	.224±.060	3.7	57:1	10.02±2.7	.393±.139	2.8	19:1	11.46±4.7
021	.419±.084	4.4	132:1	18.75±4.3	.795±.137	5.8	610:1	23.16±4.0
022	.408±.101	4.0	83:1	18.52±4.6	.873±.180	4.8	198:1	25.62±5.3
024	.457±.072	4.3	989:1	21.36±3.4	1.150±.168	6.8	1,866:1	33.74±5.0
040	.363±.076	4.8	198:1	10.45±4.0	.004±.105	.04	<1:1	1.26±31.5
041	.673±.086	7.8	4,999:1	37.41±4.8	.940±.152	6.2	908:1	32.29±5.2
042	.683±.082	8.3	>9,999:1	37.28±4.5	.939±.162	5.8	610:1	33.02±5.7
044	.776±.115	6.7	1,799:1	43.50±6.5	1.322±.144	9.2	>9,999:1	47.62±5.2
100	.087±.082	1.1	3:1	5.51±5.0	.252±.212	1.2	3:1	9.74±8.1
101	-.232±.124	1.9	7:1	-12.41±6.5	.061±.226	.3	1	2.09±7.0
102	.037±.095	.4	1:1	1.89±4.7	.849±.123	6.9	1,932:1	28.22±4.1
104	-.094±.080	1.2	3:1	-4.70±3.0	.242±.170	1.4	4:1	7.70±5.5
110	.241±.053	4.6	151:1	12.62±2.7	.430±.106	4.1	83:1	14.71±3.6
111	.178±.068	2.6	16:1	9.07±3.5	-.056±.087	.6	2	-1.77±3.0
112	.598±.092	6.5	1,221:1	34.73±5.3	1.145±.144	8.0	4,999:1	42.34±5.3
114	.612±.080	7.6	3,332:1	38.54±5.1	.920±.098	9.4	>9,999:1	35.91±3.8
120	.526±.070	7.5	3,332:1	28.93±3.9	.422±.100	4.2	99:1	13.91±3.3
121	.626±.086	7.3	3,332:1	34.47±4.7	.826±.177	4.7	169:1	26.06±5.7
122	.776±.142	5.5	400:1	44.47±5.1	1.056±.200	5.3	339:1	34.30±6.5
124	.532±.090	5.9	666:1	31.31±5.3	1.057±.185	5.7	553:1	36.24±6.4
140	.594±.121	4.9	224:1	30.12±6.1	.733±.155	4.7	179:1	23.09±4.9
141	.651±.125	5.2	323:1	32.76±6.3	1.100±.178	6.2	948:1	35.46±5.7
142	.797±.115	6.9	1,932:1	40.11±5.8	1.503±.138	10.9	>9,999:1	48.45±4.4
144	.971±.134	7.2	3,332:1	55.58±7.7	1.919±.243	7.9	4,999:1	68.36±8.6
200	-.051±.038	1.3	2:1	-2.91±2.2	-.125±.076	1.6	5:1	-4.86±3.0
201	-.147±.038	3.9	66:1	-7.17±1.8	-.168±.088	1.9	7:1	-5.25±2.8
202	-.054±.097	.6	2:1	-2.91±4.8	-.006±.177	.03	<1:1	-2.60±6.6
204	.092±.105	.9	2:1	5.32±5.9	.656±.159	4.1	88:1	24.66±6.0
210	.091±.100	.9	2:1	4.96±5.5	.029±.144	0.2	1:1	1.01±5.0
211	.264±.092	2.9	21:1	14.45±5.0	.338±.105	3.2	31:1	10.04±3.1
212	.545±.106	5.1	293:1	31.95±6.3	.577±.185	3.1	28:1	19.79±6.4
214	.447±.093	4.8	198:1	25.10±5.2	.898±.132	6.8	1,799:1	29.81±4.4
220	.543±.083	6.5	1,332:1	31.60±4.8	.227±.133	1.7	6:1	7.74±4.6
221	.593±.098	6.1	811:1	31.92±5.2	.809±.138	5.8	638:1	27.78±4.8
222	.598±.102	5.8	666:1	29.93±5.2	1.129±.136	8.3	>9,999:1	38.09±4.7
224	.371±.130	2.8	17:1	16.50±5.9	1.430±.180	8.0	2,189:1	42.88±5.4
240	.648±.091	7.1	1,469:1	29.16±4.1	1.241±.175	7.1	1,499:1	38.06±5.4
241	.571±.107	5.3	354:1	28.20±4.8	1.098±.194	5.5	431:1	33.20±6.0
242	.563±.160	3.5	43:1	25.66±7.3	1.398±.228	6.1	860:1	40.71±6.7
244	.622±.070	8.9	9,999:1	27.27±3.1	1.648±.165	10.0	>9,999:1	52.05±5.2
400	-.107±.057	1.9	6:1	-4.85±2.6	-.053±.084	.6	2:1	-1.58±2.6
401	.327±.063	5.2	195:1	17.38±3.3	.506±.132	3.8	48:1	16.63±4.4
402	-.060±.086	.7	2:1	-3.01±4.3	.188±.139	1.4	4:1	6.26±4.5
404	-.050±.086	.6	2:1	-2.24±3.7	-.089±.169	.5	2:1	-2.37±4.7
410	.287±.095	3.0	25:1	14.81±4.9	-.053±.302	.2	<1:1	-1.55±7.8
411	.318±.103	3.1	27:1	16.06±5.1	.595±.137	4.3	109:1	18.05±4.2
412	.250±.067	3.7	57:1	12.14±3.3	.693±.180	3.8	63:1	19.45±5.1
414	.572±.130	4.4	123:1	30.12±7.0	1.007±.201	5.0	241:1	29.89±6.0
420	.736±.083	8.9	>9,999:1	38.98±4.4	.946±.183	5.2	308:1	31.56±6.1
421	.422±.131	3.2	31:1	22.35±7.0	.409±.184	2.2	10:1	13.65±6.2
422	.587±.058	10.1	>9,999:1	32.04±3.2	.995±.177	7.8	4,999:1	35.15±4.5
424	.651±.046	14.2	>9,999:1	35.53±2.5	1.155±.132	8.8	>9,999:1	40.80±4.6
440	.360±.092	3.9	68:1	15.73±4.0	.895±.176	5.4	400:1	26.32±4.9
441	.571±.063	9.1	>9,999:1	24.04±2.7	1.270±.216	5.9	666:1	34.85±5.9
442	.394±.080	4.2	99:1	13.13±3.1	.940±.115	8.2	4,999:1	21.74±2.6
444	.144±.074	1.9	7:1	5.89±3.1	.822±.152	5.4	369:1	10.01±3.5

METHODS OF CRITICAL ANALYSIS

In even the most elementary types of field experiments it is customary to apply nitrogen, phosphorus, and potash singly, any two together, or all three together in various proportions. As a rule, the only use which is made of the results thus secured is to show the effect of omitting one or two of the elements. This is of considerable value

in determining the soil requirements, but no advantage is taken of the fact that salts applied singly or by twos afford a means of determining indirectly the effects of fertilizer combinations if the proper comparisons are made.

A very simple theoretical experiment will serve as an example. Assuming that the experiment consists of applications in equal amounts of 2-12-6, 0-12-6, 2-0-6, and 2-12-0, the appropriate subtractions of the yields will show the effect of each element. Thus 0-12-6 subtracted from 2-12-6, 2-0-6 subtracted from 2-12-6, and 2-12-0 subtracted from 2-12-6 will show the effect of adding nitrogen, phosphorus, and potash, respectively. In this paper the differences thus obtained are called "efficiency factors," and the respective differences are the nitrogen-efficiency factor, the phosphorus efficiency factor, and the potash efficiency factor. These factors, however, have but little value in an experiment of only four plots; but if two more plots are added and the same amounts of 4-12-6 and 8-12-6 are used, the efficiency factors can then be utilized. Thus by subtracting the yields from treatment 0-12-6 from those of 2-12-6, 4-12-6, and 8-12-6, the respective efficiencies of 2, 4, and 8 per cent nitrogen can be determined. The relationship is very simple, and the efficiency of the nitrogen applications varies directly with the yield, since the yield of 0-12-6 is a constant. The relationship, however, may be much more complex. Let it be assumed that four treatments of a different type are added to the original experiment, as for instance, 0-18-6, 0-24-6, 2-18-6, and 2-24-6, and that the nitrogen efficiency of 2-12-6, 2-18-6, and 2-24-6 is desired. This may be obtained by subtracting from the preceding the treatments 0-12-6, 0-18-6, and 0-24-6, respectively. Should the efficiency of nitrogen increase in the order in which the treatments appear, it would be concluded that successively larger dosages of phosphorus tend to increase the efficiency of nitrogen. This is an indirect effect due to phosphorus which can not be determined by direct inspection of the yields for each treatment. This method can be extended to include all three nutrient elements and, moreover, proves to be useful in analyzing plot data.

The problem analyzed in this paper is based on a 6-year accumulation of sweet-corn yields from 389 separate plots in a fertilizer-rotation series at Urbana, Ill. The means in Table 1 have been calculated from approximately 1,200 separate plot records and constitute the problem in the solution of which the efficiency-factor method will be applied.

LOCATION AND PLAN OF THE EXPERIMENTS

The experiment was laid out in four 10-acre fields at Urbana, Ill. Three of the fields had been planted for an indefinite period to the field corn and oats rotation typical of central Illinois, but the fourth field was in bluegrass pasture for four years prior to the experiment. Neither limestone nor commercial fertilizers of any kind had ever been applied to any of the fields. Soil-acidity tests of several types showed a limestone requirement of 3 tons, and this was applied at the beginning of the experiment. The soils in these fields vary very widely, but the predominating one is a dark semimature type having a noncalcareous subsoil.

The four fields were first laid out in 1922 and 1923 on the basis of a 4-year rotation consisting of wheat or oats, red clover, followed by

two crops of sweet corn grown for canning, which was a typical rotation at that time. Fertilizer was applied to each of the two sweet-corn crops, the yields of which are the only ones presented in this paper.

The field methods used throughout the work were as accurate as could be devised. All the fertilizers were broadcast by hand and harrowed in within 24 hours of planting. The corn was planted rather thickly by hand in check rows 42 by 42 inches and later thinned to three stalks per hill. The variety of sweet corn used was Country Gentlemen, bred at the station.

The fertilizer treatments consisted of a modification of those recommended by Spillman (8).² The following arbitrary dosages were selected as the basis of application:

7.5, 15, and 30 pounds nitrogen per acre as sodium nitrate.
32, 64, and 128 pounds P_2O_5 per acre as 16 per cent superphosphate.
25, 50, and 100 pounds K_2O per acre as potassium chloride.

These treatments are called respectively, single, double, and quadruple, and will frequently be referred to in the text as such. Whereas Spillman (8) recommended a single and double treatment, this plan was elaborated by adding a quadruple treatment.

These quantities of plant nutrients were applied in all possible mathematical combinations. Excluding the zero dosage for all 3 elements (checks), there are 63 combinations. An advantage of this layout is the large number of possible direct comparisons. There are 48 possible comparisons for determining the effects of any one of the three single elements, 16 where any two vary, and 4 where all three vary.

For the sake of brevity the treatments will be presented in the form of the amount applied in the conventional N-P-K sequence. Thus the treatment 124 means a single dosage of nitrogen (7.5 pounds per acre), a double dosage of phosphorus (64 pounds P_2O_5 per acre), and a quadruple dosage of potash (100 pounds K_2O per acre). In the case of 024, the dosage is the same as 124 except that the nitrogen is omitted. A complete list of the treatments follows:

000	020	100	120	200	220	400	420
001	021	101	121	201	221	401	421
002	022	102	122	202	222	402	422
004	024	104	124	204	224	404	424
010	040	110	140	210	240	410	440
011	041	111	141	211	241	411	441
012	042	112	142	212	242	412	442
014	044	114	144	214	244	414	444

The plots were so laid out in each field that every third one was a check. No maintenance fertilizer treatment was applied to any of the checks during the period covered by this report. Nevertheless, the check yields have been maintained reasonably well by the use of limestone and clover. The treated plots differ from the checks in receiving only the fertilizer in addition to limestone and clover. The practice has been to remove from all plots, including checks, one cutting of clover, one small-grain crop, including the straw, and only the green ears from the two sweet-corn crops. The green fodder was cut, weighed, and then returned to the plot from which it came.

² Reference is made by number (italic) to Literature Cited, p. 704.

All the subsequent data have been computed on an acre basis from the plots, each of which is 28 by 77 feet, or $0.04949 +$ acre, net size after a single border row had been removed around the plot. The gross size is 31.5 by 80.5 feet, or 0.058213 acre. The distance between the net plots with borders removed is 15 feet in each field.

All the fertilizer salts used in this experiment were chemically analyzed, and the amounts applied to each plot were calculated on this basis.

EXPERIMENTAL METHODS

The harvesting of sweet corn in the edible green stage presents problems peculiar to this crop. Botanically immature when snapped, sweet corn is accordingly subject to considerable variation in total weight of ears due to increases in the amount of food material stored and the total percentage of moisture present at different stages. Wide variations in maturity resulting from the differential effect of fertilizers prevent any possibility of harvesting the plots simultaneously without incurring a large and decisive experimental error due to differences in weight. On the basis of the work of Appleman and Eaton (3) the author experimented in 1922 with a method of silk counting in order to determine the probable date of harvest for each plot. Appleman (2) and Culpepper and Magoon (4) in extensive experiments with sweet corn, have shown that maturity can be determined with reasonable accuracy by means of silk counts. The accuracy of the method with respect to field corn has been demonstrated by Myers (7) who has shown that there is a high degree of correlation between silk counts and dry maturity. Although there is reason to believe that silk counting in sweet corn is a much less reliable index of maturity than in field corn, owing to the multiplicity of suckers and shoots in the former, no other field method of predicting maturity is known at present which will answer the same purpose.

The weights given in the subsequent tables are for edible green corn harvested as nearly as possible at a stage when not more than 5 per cent of the ears by weight were showing slight denting. The weights are those of sorted corn, the culls being omitted.

The weights of green fodder were obtained by weighing the stalks immediately after the green corn was harvested.

No corrections for soil variations have been made beyond comparing the yield of each treatment with that of the adjacent check.

STATISTICAL METHODS

Each treatment has been paired with its respective adjacent check and the significance of the mean differences in yield determined by the use of Student's method (1, 5, 6). The odds appear in Table 1.

The probable errors of the mean increases in yields expressed both as tons and percentages have been calculated according to the formula $\frac{0.6745 S. D.}{\sqrt{n}}$. The probable errors of certain factors appearing in

Tables 2 to 13, inclusive, have also been determined, and the methods of calculation will be discussed along with the derivation of these factors. Deviations which are 3.2 or more times their probable errors are considered statistically significant. Student's odds which are 30:1 or more are likewise considered significant.

EXPERIMENTAL RESULTS

The mean increases or decreases in yield from the fertilized plots as compared with the yields from the respective adjacent checks, together with the odds and the respective percentages, are shown in Table 1. These means have been calculated from 12 annual crops over the 6-year period, 1923 to 1928, inclusive. The data in Tables 2 to 7 have been computed from Table 1, but the intermediate steps are not shown.

The results are discussed from two standpoints, the first in terms of increased yields expressed as percentages and taken directly from Table 1, and the second as efficiency factors. The term "efficiency factor" as used in this paper means the increase in the percentage yield due to one of the three major nutrient elements designated. Each efficiency factor has been calculated from Table 1 by means of direct comparisons between appropriate fertilizer combinations. For example, in treatment 144 it is possible to determine for edible green ears the efficiency factors for all three elements, as follows:

Nitrogen efficiency factor:

Per cent increase in yield, treatment 144.....	55.58 ± 7.7
Per cent increase in yield, treatment 044.....	43.50 ± 6.5

Increase due to nitrogen, or N efficiency factor..... 12.08 ± 10.1

Phosphorus efficiency factor:

Per cent increase in yield, treatment 144.....	55.58 ± 7.7
Per cent increase in yield, treatment 104.....	-4.70 ± 3.9

Increase due to phosphorus, or P efficiency factor..... 60.28 ± 8.6

Potash efficiency factor:

Per cent increase in yield, treatment 144.....	55.58 ± 7.7
Per cent increase in yield, treatment 140.....	30.12 ± 6.1

Increase due to potash, or K efficiency factor..... 25.46 ± 9.8

The probable errors of the efficiency factors have been calculated from the formula $\sqrt{a^2 + b^2}$ where a and b represent the probable errors of the two percentages used in the subtraction. The correlation is assumed to equal zero.

Because of the mathematical relation existing between the fertilizer treatments, a very large number of comparisons may be made. In order to avoid confusion, a uniform method of analysis is used in Tables 2 to 7, which consists of determining the efficiency factors for individual treatments in the appropriate 2-element series and comparing them by means of curves (figs. 1, A, to 12, A, and 1, C, to 12, C) derived from Tables 2 to 7. Comparisons must also be made, of course, in the 3-element series, that is, the complete fertilizers, but as there are three dosages of the third element in each case, three additional sets of curves would be necessary. In order to avoid this, the 0, 1, 2, and 4 dosages of the third or quiescent element, have been averaged in Tables 2 to 7 and these means compared in curves. (Figs. 1, B, to 12, B, and 1, D, to 12, D.) In order to indicate the fact that a given treatment is a mean of this type, the letter X is substituted for the third or quiescent element. Thus 12X indicates that this treatment is the average of 120, 121, 122, and 124.

The probable errors of these "X" treatments are determined in each instance by means of the formula $\frac{1}{N}\sqrt{a^2 + b^2 + c^2} \dots n^2$, in which

a, *b*, *c*, etc., indicate the probable errors of single deviations, and *N* the number of deviations, which is four in each case.

This system of averaging is open to certain objections, the principal one being that comparisons between individual treatments may lead to conclusions which differ from those in this paper. The same criticism is applicable, however, whenever any attempt is made to compare the trends between single determinations and means.

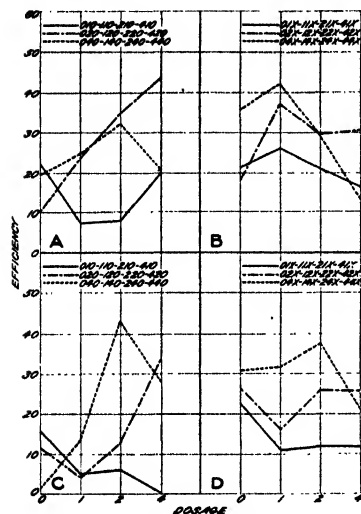


FIGURE 1.—The effect of successive increments of nitrogen on the efficiency of phosphorus, both with and without potash; results for two elements shown in A and C, for three elements in B and D, for edible ears in A and B, and for green fodder in C and D

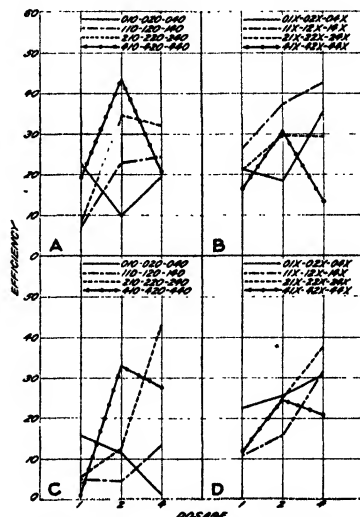


FIGURE 2.—The effect of successive increments of phosphorus on its efficiency in nitrogen-phosphorus combinations, both with and without potash; results for two elements shown in A and C, for three elements in B and D, for edible ears in A and B, and for green fodder in C and D

EFFECT OF NITROGEN ON THE EFFICIENCY OF PHOSPHORUS

The effect of nitrogen on the efficiency of phosphorus is shown in Table 2 and Figures 1 and 2. The efficiency factors for both edible ears and green fodder are given.

The efficiency factors for edible ears (Table 2 and figs. 1, A, and 1, B) will be discussed first, comparisons being made in the order of increasing nitrogen with phosphorus constant, as follows:

010-110-210-410.
020-120-220-420.
040-140-240-440.

Where the mean factors are compared, X is simply substituted for the "0" potash. In Table 2 and Figure 1, A, the efficiency factors of 020-120-220-420, all of which are significant, appear in a constantly increasing order. In the single phosphorus series (010-110-210-410) nitrogen apparently has a depressing effect, although only one of the nitrogen treatments is statistically significant. A comparison of the efficiency factors in the series 040-140-240-440 shows an upward tendency in phosphorus efficiency reaching the maximum in treatment 240.

The mean efficiency factors in Figure 1, B, may be similarly compared. All these factors are statistically significant, according to Table 2. With low phosphorus constant in series 01X-11X-21X-41X, nitrogen in larger quantities than the single dosage exercises a depressing effect. This tendency also appears in the nitrogen-phosphorus combinations (010-110-210-410), but the curves do not resemble each other very closely. The series 02X-12X-22X-42X and 04X-14X-24X-44X resemble each other in the increased efficiency shown by the single dosage of nitrogen, followed by a decline when heavier treatments are applied. There is no resemblance between these two curves in Figure 1, B, and those in Figure 1, A, for the series containing equivalent amounts of nitrogen and phosphorus.

TABLE 2.—Comparison of the effect of nitrogen on the mean percentage increases in yields of edible green sweet corn and of green sweet-corn fodder, and on the efficiency of phosphorus, basis of percentages

GREEN SWEET CORN

Treat-ment	Increase in yield	P efficiency factor	Treat-ment	Increase in yield	P efficiency factor	Treat-ment	Increase in yield	P efficiency factor
010....	22.63±4.8	22.63±4.8	020....	10.02±2.7	10.02±2.7	040....	19.45±4.0	19.45±4.0
011....	13.91±4.0	22.89±4.8	021....	18.75±4.3	27.73±5.1	041....	37.41±4.8	46.39±5.5
012....	17.99±5.0	16.36±5.2	022....	18.62±4.6	16.89±4.9	042....	37.28±4.5	35.65±4.8
014....	27.62±5.4	24.04±6.3	024....	21.36±3.4	17.78±4.7	044....	43.50±6.5	39.92±7.2
Mean...	20.54±2.4	21.48±2.7	Mean...	17.16±1.5	18.10±2.0	Mean...	34.41±2.5	35.35±2.8
110....	12.62±2.7	7.11±5.7	120....	28.93±3.6	23.42±6.3	140....	30.12±6.1	24.61±7.9
111....	9.07±3.5	21.48±7.4	121....	34.47±4.7	46.88±6.8	141....	32.76±6.3	45.17±9.0
112....	34.73±5.3	32.84±7.1	122....	44.47±8.1	42.58±9.4	142....	40.11±5.8	38.22±7.5
114....	38.54±5.1	43.24±6.4	124....	31.11±5.5	35.81±6.6	144....	55.68±7.7	60.28±8.6
Mean...	23.74±2.1	26.17±3.3	Mean...	34.74±2.1	37.17±3.7	Mean...	39.64±3.3	42.07±4.1
210....	4.96±5.5	7.87±5.9	220....	31.50±4.1	34.41±5.3	240....	29.16±4.1	32.07±4.6
211....	14.45±5.0	21.62±5.3	221....	31.92±5.2	39.09±5.5	241....	26.20±4.8	33.37±5.1
212....	31.95±6.3	34.86±7.9	222....	29.93±5.2	32.84±7.1	242....	25.66±7.3	28.57±8.7
214....	25.10±5.2	19.78±7.9	224....	16.50±5.9	11.18±8.3	244....	27.27±3.1	21.95±6.7
Mean...	19.12±2.8	21.03±3.4	Mean...	27.46±2.6	29.38±3.3	Mean...	27.07±2.5	28.99±3.2
410....	14.81±4.9	19.66±5.5	420....	38.98±4.4	43.83±5.1	440....	15.73±4.0	20.68±4.8
411....	16.06±5.1	-1.32±6.1	421....	22.35±7.0	4.97±7.7	441....	24.94±2.7	7.56±4.3
412....	12.14±3.3	15.15±5.4	422....	32.04±3.2	35.06±5.4	442....	13.13±3.1	16.14±5.3
414....	30.12±7.0	32.36±7.9	424....	35.53±2.5	37.77±4.5	444....	5.89±3.1	8.13±4.8
Mean...	18.28±2.6	16.46±3.1	Mean...	32.22±2.3	30.40±2.9	Mean...	14.92±1.6	13.10±2.4

GREEN SWEET-CORN FODDER

010....	15.64±4.0	15.64±4.0	410....	-1.55±7.8	0.08±8.2	220....	7.74±4.6	12.60±5.5
011....	10.97±3.8	23.21±8.1	411....	18.05±4.2	1.42±6.1	221....	27.78±4.8	33.03±5.6
012....	13.58±4.4	8.51±5.6	412....	19.45±5.1	13.19±6.8	222....	38.69±4.7	38.89±8.1
014....	39.36±5.0	41.80±6.1	414....	29.89±6.0	32.26±7.6	224....	42.88±5.4	18.22±8.1
Mean...	19.89±2.2	22.29±3.1	Mean...	16.46±3.0	11.72±3.6	Mean...	29.27±2.4	25.68±3.5
110....	14.71±3.6	4.97±8.9	020....	11.46±4.1	11.46±4.1	420....	31.56±6.1	33.14±6.6
111....	-1.77±3.0	-3.86±7.6	021....	23.18±4.0	35.42±8.2	421....	13.65±6.2	-2.98±7.6
112....	42.34±5.3	14.12±6.7	022....	25.62±5.3	20.65±6.3	422....	35.15±4.5	28.89±6.4
114....	35.91±3.8	28.21±6.7	024....	33.74±5.0	36.18±6.1	424....	40.80±4.6	43.17±6.6
Mean...	22.80±2.0	10.86±3.8	Mean...	23.50±2.3	25.90±3.2	Mean...	30.29±2.7	25.56±3.4
210....	1.01±5.0	5.87±5.8	120....	13.91±3.3	4.17±8.7	040....	1.26±31.5	1.26±31.5
211....	10.04±3.1	15.29±4.2	121....	26.66±5.7	24.57±9.0	041....	32.29±5.2	44.53±8.9
212....	19.79±6.4	19.99±9.2	122....	34.30±6.5	6.08±7.7	042....	33.02±5.7	27.95±6.6
214....	29.81±4.4	5.15±7.4	124....	36.24±6.4	28.54±8.4	044....	47.62±5.2	50.06±6.3
Mean...	15.16±2.4	11.58±3.4	Mean...	27.78±2.8	15.84±4.2	Mean...	28.55±8.2	30.95±8.5

TABLE 2.—*Comparison of the effect of nitrogen on the mean percentage increases in yields of edible green sweet corn and of green sweet-corn fodder, and on the efficiency of phosphorus, basis of percentages—Continued*

GREEN SWEET-CORN FODDER—Continued

Treat- ment	Increase in yield	P efficiency factor	Treat- ment	Increase in yield	P efficiency factor	Treat- ment	Increase in yield	P efficiency factor
140.	23.09± 4.9	13.35± 9.5	240.	38.06± 5.4	42.92± 6.2	440.	26.32± 4.9	27.90± 5.5
141.	35.46± 5.7	33.37± 9.0	241.	33.20± 6.0	38.45± 6.6	441.	34.85± 5.9	18.22± 7.4
142.	48.45± 4.4	20.23± 6.0	242.	40.71± 6.7	40.91± 9.4	442.	21.74± 2.6	15.48± 5.2
144.	68.36± 8.6	60.66±10.2	244.	52.05± 5.2	27.39± 7.9	444.	19.01± 3.5	21.38± 5.9
Mean..	43.84± 3.1	31.90± 4.4	Mean..	41.00± 2.9	37.42± 3.8	Mean..	25.48± 2.2	20.74± 3.0

The effect of nitrogen as determined by green fodder is shown in Table 2 and Figure 1, C and D. The depressive effect of nitrogen upon phosphorus in the series 010-110-210-410 is rather marked. The tendency shown here is similar to that found in edible ears. In the two series 020-120-220-420 and 040-140-240-440, nitrogen has a variable but, in some dosages, stimulating effect.

The mean factors in Figure 1, D, are all statistically significant with the exception of treatment 11X. (Table 2.) Again there appears the depressive effect of nitrogen upon the single dosage of phosphorus (01X-11X-21X-41X). Nitrogen in the series 02X-12X-22X-42X and 04X-14X-24X-44X has but little stimulating effect and a considerably depressive one in certain treatments (12X and 44X).

No consideration has been given thus far to the efficiency of phosphorus when this element is applied in increasing amounts with nitrogen held constant. Such comparisons are made in Figure 2 and may be summarized as follows:

010-020-040.
110-120-140.
210-220-240.
410-420-440.

The letter X is simply substituted in place of the 0 potash when the mean factors are compared.

On the basis of edible ears (fig. 2, A) the double phosphorus dosage is consistently more efficient than any other in combination of nitrogen and phosphorus. At the same time it is statistically significant in all cases in Figure 2, A. Phosphorus applied alone gives rather unexpected results inasmuch as the single dosage is the most efficient.

A comparison of the mean factors in Figure 2, B, shows that where nitrogen is used the double phosphorus dosage is the most efficient in two cases out of three, and in the third, 12X is only slightly inferior to 14X. Omitting nitrogen (01X-02X-04X) gives an irregular trend, 04X being more efficient than any other combination in the series.

Comparisons of the effect of the dosage of phosphorus on the efficiency factors for fodder are shown in Figure 2, C and D, which have been derived from Table 2. As in the case of edible ears, phosphorus applied alone has a tendency to decrease in efficiency as the size of the application increases. When phosphorus is combined with nitrogen, however, there is a general tendency for its efficiency to increase in relation to the dosage, with the single exception of treatment 120.

The mean efficiency factors show a similar trend (fig. 2, D) when nitrogen is present, that is, the efficiency tends to increase in relation to the amount applied. Omitting nitrogen, as in series 01X-02X-04X, fails to change this trend.

From the preceding discussion it is apparent that under certain conditions in these experiments, nitrogen may have a considerable influence upon the efficiency of phosphorus. With a dosage of 32 pounds P_2O_5 per acre constant, nitrogen seems to act in general as a depressive agent. On the other hand, with 64 or 128 pounds P_2O_5 per acre constant, there is a general tendency for nitrogen in treatments of 7.5 and 15 pounds per acre to increase the efficiency of phosphorus. Edible ears are somewhat more consistent in their response than is fodder, according to Figure 1, C and D.

The efficiency of phosphorus changes considerably according to the amount applied. In combinations containing nitrogen but no potash there is a marked tendency for phosphorus to increase in efficiency in relation to the amount applied. This is in striking contrast with the effect shown by phosphorus applied alone, where the increased dosage gives a decreased efficiency. A comparison of edible ears with fodder shows that the double application of phosphorus is as efficient as the quadruple application on the edible-ear basis, or more so, but on the fodder basis the reverse is true.

The trends shown by the mean efficiency factors differ in some instances from those of the nitrogen-phosphorus combinations. In the former, the single dosage of nitrogen, using edible ears as a basis, is the most efficient, whereas in the latter heavier nitrogen applications seem to be superior. A comparison of the trends shown by the two series 010-020-040 and 01X-02X-04X indicates that potash is also an important factor in influencing the efficiency of phosphorus. This will be discussed later.

EFFECT OF PHOSPHORUS ON THE EFFICIENCY OF NITROGEN

The data showing the effect of phosphorus on the efficiency of nitrogen appear in Table 3 and in Figures 3 and 4.

Considering first the results obtained from edible ears (Table 3 and fig. 3, A and B) it is evident that the single dosage of phosphorus has a slight to depressing effect on nitrogen efficiency. The results, however, are not statistically significant. The double phosphorus dosage has a uniformly distinct and significant stimulating effect on nitrogen.

A study of the results from fodder (Table 3 and fig. 3, C and D) shows that the single phosphorus dosage is depressive, whereas in general the double and quadruple dosages are stimulating. Since almost all the factors lack statistical significance, this can only be considered as a trend.

The curves in Figure 4, A and B, show the effect of increasing dosages of nitrogen upon its own efficiency (edible-ear basis), phosphorus being held constant. The only factors in Figure 4, A, having statistical significance are in the series 120-220-420; in this series nitrogen increases somewhat in efficiency. The remaining curves of Figure 4, A, show that increasing the dosages of nitrogen tends to reduce its efficiency.

In Figure 4, B, the series 12X-22X-42X, in which the factors are statistically significant, shows a somewhat variable but slightly downward trend. The remaining curves, with the exception of 10X-20X-40X, show that nitrogen has a tendency to decrease in efficiency as the dosage increases.

The curves for fodder (fig. 4, C and D) are derived from Table 3. • In Figure 4, C, there is a good deal of variation. Thus in the series 100-200-400 and 110-210-410 increasing the nitrogen tends to decrease its efficiency. With larger amounts of phosphorus constant, nitrogen shows a partially reverse tendency. The mean factors in Figure 4, D, are much more consistent, and nitrogen in increasing amounts seems either to have no appreciable effect upon its

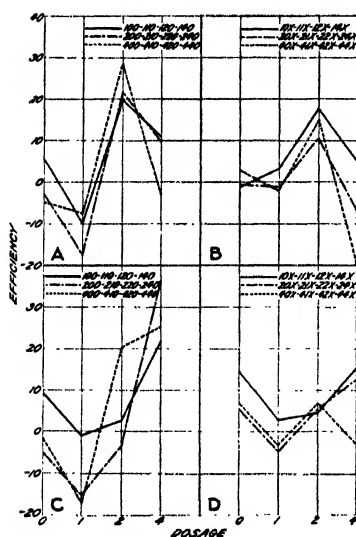


FIGURE 3.—The effect of successive increments of phosphorus on the efficiency of nitrogen, both with and without potash; results for two elements shown in A and C, for three elements in B and D, for edible ears in A and B, and for green fodder in C and D

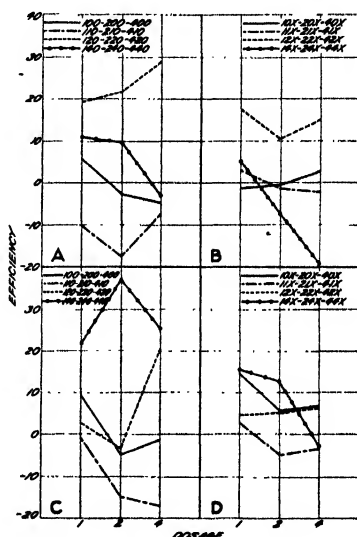


FIGURE 4.—The effect of successive increments of nitrogen on its efficiency in nitrogen-phosphorus combinations, both with and without potash; results for two elements shown in A and C, for three elements in B and D, for edible ears in A and B, and for green fodder in C and D

efficiency, or a depressive one. These results are to be regarded only as tendencies, however, since but one of the efficiency factors is statistically significant.

The discussion of the effect of phosphorus on nitrogen efficiency emphasizes the importance of the double dosage of phosphorus in the production of edible ears, inasmuch as it increases the efficiency of nitrogen to a significant degree. On the other hand, applications of 32 pounds P_2O_5 per acre have a depressive effect as compared with no phosphorus. The results from fodder agree with the foregoing to the extent of confirming the depressive tendency of the single phosphorus dosage, but the quadruple phosphorus dosage is in general more efficient than the double one.

The curves which interpret the effect of the dosage of nitrogen upon its efficiency show that in most instances, except those noted above, the single dosage is as efficient as the heavier applications, or more so. Thus, the analysis brings out two points of importance, namely, that the single dosage of nitrogen and the double dosage of phosphorus appear to be the most efficient, the latter from the standpoint of producing edible ears.

TABLE 3.—Comparison of the effect of phosphorus on the mean percentage increases in yields of edible green sweet corn and of sweet-corn fodder, and on the efficiency of nitrogen, basis of percentages

EDIBLE GREEN SWEET CORN

Treat- ment	Increase in yield	N efficiency factor	Treat- ment	Increase in yield	N efficiency factor	Treat- ment	Increase in yield	N efficiency factor
100....	5.51±5.0	5.51± 5.0	200....	-2.91±2.2	-2.91± 2.2	400....	-4.85±2.6	-4.85± 8.1
101....	-12.41±6.5	-3.43± 7.0	201....	-7.17±1.8	1.81± 3.2	401....	17.38±3.3	26.36± 4.3
102....	1.89±4.7	.26± 4.9	202....	-2.91±4.8	-4.54± 5.0	402....	-3.01±4.3	-4.64± 4.6
104....	-4.70±3.9	-8.28± 5.0	204....	5.32±5.9	1.74± 6.7	404....	-2.24±3.7	-5.82± 4.9
Mean.	-2.43±2.6	-1.49± 2.8	Mean.	-1.92±2.0	-.98± 2.3	Mean.	1.82±1.8	2.70± 2.8
110....	12.62±2.7	-10.01± 5.5	210....	4.96±5.5	-17.67± 7.3	410....	14.81±4.9	-7.82± 6.8
111....	9.07±3.5	-4.84± 5.3	211....	14.45±5.0	.54± 6.4	411....	16.06±5.1	2.15± 6.5
112....	34.73±8.1	16.74± 7.3	212....	31.95±6.3	13.96± 8.0	412....	12.14±3.3	-5.85± 6.0
114....	38.54±5.1	10.92± 7.4	214....	25.10±5.2	-2.52± 7.5	414....	30.12±7.0	2.50± 8.8
Mean.	23.74±2.1	3.20± 3.2	Mean.	19.12±2.8	-1.42± 3.7	Mean.	18.28±2.6	-2.26± 3.6
120....	28.03±3.9	18.91± 4.7	220....	31.50±4.8	21.48± 5.5	420....	38.98±4.4	28.96± 5.2
121....	34.47±4.7	15.72± 6.4	221....	31.92±3.2	13.17± 6.5	421....	22.35±7.0	3.60± 8.2
122....	44.47±8.1	25.95± 9.3	222....	29.03±5.2	11.41± 6.9	422....	32.04±3.2	13.52± 5.6
124....	31.11±5.3	9.75± 6.3	224....	16.50±5.9	-4.86± 6.8	424....	35.53±2.5	14.17± 4.2
Mean.	34.74±2.9	17.68± 3.4	Mean.	27.46±2.6	10.30± 3.2	Mean.	32.22±2.3	15.06± 3.0
140....	30.12±6.1	10.67± 7.3	240....	29.16±4.1	9.71± 5.7	440....	15.73±4.0	-3.72± 5.6
141....	32.76±6.3	-4.05± 7.9	241....	26.20±4.8	-11.21± 6.8	441....	24.64±2.7	-12.47± 5.5
142....	40.11±5.8	2.83± 7.3	242....	25.66±7.3	-11.62± 8.6	442....	13.13±3.1	-24.15± 5.5
144....	55.58±7.7	12.06±10.1	244....	27.27±3.1	-16.23± 7.2	444....	5.89±3.1	-37.61± 7.2
Mean.	39.64±3.3	5.23± 4.1	Mean.	27.07±2.5	-7.34± 3.6	Mean.	14.92±1.6	-19.49± 3.0

SWEET CORN FODDER

Treat- ment	Increase in yield	N efficiency factor	Treat- ment	Increase in yield	N efficiency factor	Treat- ment	Increase in yield	N efficiency factor
100....	9.74±8.1	9.74± 8.1	200....	-4.86±3.0	-4.86± 3.0	400....	-1.58±2.6	-1.58± 2.6
101....	2.09±7.0	14.33±10.0	201....	-5.25±2.8	6.99± 2.9	401....	16.63±4.4	28.87± 4.4
102....	28.22±4.1	23.15± 5.3	202....	-20.6±6.6	-5.27± 7.4	402....	6.26±4.5	1.19± 5.6
104....	7.70±5.5	10.14± 6.5	204....	24.66±6.0	27.10± 6.9	404....	-2.37±4.7	.07± 5.9
Mean.	11.94±3.2	14.34± 3.8	Mean.	3.59±2.4	5.99± 2.7	Mean.	4.74±2.1	7.14± 2.4
110....	14.71±3.6	-.93± 5.4	210....	1.01±5.0	-14.63± 6.4	410....	-1.55±7.8	-17.19± 8.8
111....	-1.77±3.0	-12.74± 4.8	211....	10.04±3.1	-.93± 4.9	411....	18.05±4.2	7.06± 5.7
112....	42.34±5.3	28.76± 6.9	212....	19.79±6.4	6.21± 7.8	412....	19.45±5.1	5.87± 6.7
114....	35.91±3.8	-3.46± 6.3	214....	29.81±4.4	-9.55± 6.7	414....	29.89±6.0	-9.47± 7.8
Mean.	22.80±2.0	2.91± 3.0	Mean.	15.16±2.4	-4.72± 3.3	Mean.	16.46±3.0	-3.43± 3.7
120....	13.91±3.3	2.45± 5.3	220....	7.74±4.6	-3.72± 6.2	420....	31.56±6.1	20.10± 7.4
121....	26.66±5.7	3.48± 7.0	221....	27.78±4.8	4.60± 6.3	421....	13.65±6.2	-9.63± 7.4
122....	34.30±6.5	8.05± 8.4	222....	38.69±4.7	13.07± 7.1	422....	35.15±4.5	9.63± 7.0
124....	36.24±6.4	2.50± 8.1	224....	42.88±5.4	9.14± 7.4	424....	40.80±4.6	7.06± 6.8
Mean.	27.78±2.8	4.28± 3.6	Mean.	29.27±2.4	5.77± 3.4	Mean.	30.29±2.7	6.79± 3.6
140....	23.09±4.9	21.83±31.9	240....	38.06±5.4	36.80±32.0	440....	26.32±4.9	25.06±31.9
141....	35.46±5.7	3.17± 7.7	241....	33.20±6.0	.91± 7.9	441....	34.85±5.9	2.56± 7.9
142....	48.45±4.4	15.45± 7.2	242....	40.71±6.7	7.69± 8.8	442....	21.74±2.6	-11.28± 6.3
144....	68.36±8.6	20.74±10.0	244....	52.05±5.2	4.43± 7.4	444....	19.01±3.5	-28.61± 6.3
Mean.	43.84±3.1	15.29± 8.8	Mean.	41.00±2.9	12.46± 8.7	Mean.	25.48±2.2	-3.07± 8.5

EFFECT OF NITROGEN ON THE EFFICIENCY OF POTASH

The effect of nitrogen on the efficiency of potash is shown in Table 4 and Figures 5 and 6.

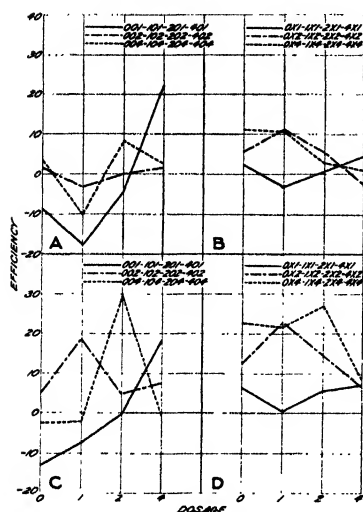


FIGURE 5.—The effect of successive increments of nitrogen on the efficiency of potash, both with and without phosphorus; results for two elements shown in A and C, for three elements in B and D, for edible ears in A and B, and for green fodder in C and D

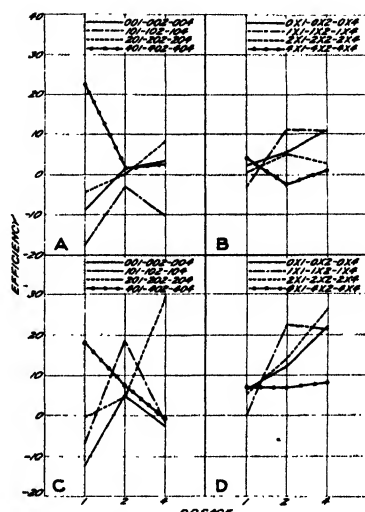


FIGURE 6.—The effect of successive increments of potash on its efficiency in nitrogen-potash combinations, both with and without phosphorus; results for two elements shown in A and C, for three elements in B and D, for edible ears in A and B, and for green fodder in C and D

TABLE 4.—Comparison of the effect of nitrogen on the mean percentage increases in yields of edible green sweet corn and of sweet-corn fodder, and on the efficiency of potash, basis of percentages

EDIBLE GREEN SWEET CORN

Treat-ment	Increase in yield	K efficiency factor	Treat-ment	Increase in yield	K efficiency factor	Treat-ment	Increase in yield	K efficiency factor
001...	-8.98±2.7	-8.98±2.7	002...	1.63±1.6	1.63±1.6	004...	3.58±3.2	3.58±3.2
011...	13.91±4.0	-8.72±6.2	012...	17.99±5.0	-4.64±6.9	014...	27.62±5.4	4.90±7.2
021...	18.75±4.3	8.73±5.1	022...	18.52±4.6	8.50±5.3	024...	21.36±3.4	11.34±4.3
041...	37.41±4.8	17.96±6.2	042...	37.28±4.5	17.83±6.0	044...	43.50±6.5	24.05±7.6
Mean...	15.27±2.0	2.25±2.6	Mean...	18.86±2.1	5.83±2.7	Mean...	24.02±2.4	10.99±2.9
101...	-12.41±6.5	-17.92±8.2	102...	1.89±4.7	-3.62±6.9	104...	-4.70±3.9	-10.21±6.3
111...	9.07±3.5	-3.55±4.4	112...	34.73±5.3	22.11±5.9	114...	38.54±5.1	25.92±5.8
121...	34.47±4.7	5.54±6.1	122...	44.47±8.1	15.54±9.0	124...	31.11±5.3	2.18±6.6
141...	32.76±6.3	2.64±8.8	142...	40.11±5.8	9.99±8.4	144...	55.58±7.7	25.46±9.8
Mean...	15.97±2.7	-3.32±3.5	Mean...	30.30±3.0	11.00±3.8	Mean...	30.13±2.8	10.81±3.6
201...	-7.17±1.8	-4.26±2.8	202...	-2.91±4.8	0	204...	5.32±5.9	8.23±6.3
211...	14.45±5.0	9.49±7.4	212...	31.95±6.3	26.99±8.4	214...	25.10±5.2	20.14±7.6
221...	31.92±5.2	5.42±7.1	222...	29.93±5.2	-1.57±7.1	224...	16.50±5.9	-15.00±7.6
241...	26.20±4.8	-2.96±6.3	242...	25.66±7.3	-3.50±8.4	244...	27.27±3.1	-1.89±5.1
Mean...	16.35±2.2	.67±3.1	Mean...	21.16±3.0	5.48±3.5	Mean...	18.55±2.6	2.87±3.4
401...	17.38±3.3	22.23±4.2	402...	-3.01±4.3	1.84±5.0	404...	-2.24±3.7	2.61±4.5
411...	16.06±5.1	1.25±7.1	412...	12.14±3.3	-2.67±5.9	414...	30.12±7.0	15.31±8.5
421...	22.35±7.0	-16.63±8.3	422...	32.04±3.2	-6.94±5.4	424...	35.53±2.5	-3.46±5.1
441...	24.94±2.7	9.21±4.8	442...	13.13±3.1	-2.60±5.1	444...	5.89±3.1	-9.84±5.1
Mean...	20.18±2.4	4.04±3.2	Mean...	13.58±1.8	-2.59±2.7	Mean...	17.32±2.2	1.16±3.0

TABLE 4.—Comparison of the effect of nitrogen on the mean percentage increases in yields of edible green sweet corn and of sweet-corn fodder, and on the efficiency of potash, basis of percentages—Continued

GREEN SWEET-CORN FODDER

Treatment	Increase in yield	K efficiency factor	Treatment	Increase in yield	K efficiency factor	Treatment	Increase in yield	K efficiency factor
001.	-12.24±7.2	-12.24± 7.2	002.	5.07±3.4	5.07± 3.4	004.	-2.44±3.5	-2.44± 3.5
011.	10.97±3.8	4.67± 5.5	012.	13.58±4.4	-2.06± 5.9	014.	39.36±5.0	23.72± 6.4
021.	23.18±4.0	11.72± 5.7	022.	25.62±5.3	14.16± 6.2	024.	33.74±5.0	22.28± 6.5
041.	32.29±5.2	31.03±31.9	042.	33.02±5.7	31.76±32.0	044.	47.62±5.2	46.36±31.9
Mean.	13.55±2.6	6.46± 8.4	Mean.	19.32±2.4	12.23± 8.4	Mean.	29.57±2.4	22.48± 8.3
101.	2.09±7.0	-7.65±10.7	102.	28.22±4.1	18.48±9.1	104.	7.70±5.5	-2.04±9.8
111.	-1.77±3.0	-16.48± 4.7	112.	42.34±5.3	27.63±6.3	114.	35.91±3.8	21.20±5.2
121.	26.66±5.7	12.76± 6.6	122.	34.30±6.5	20.39±7.3	124.	36.24±6.4	22.33±7.2
141.	35.46±5.7	12.37± 7.5	142.	48.45±4.4	25.36±6.6	144.	68.36±8.6	45.27±9.9
Mean.	15.61±2.8	.25± 3.8	Mean.	38.33±2.6	22.96±3.7	Mean.	37.05±3.2	21.69±4.1
201.	-5.25±2.8	- .39±4.1	202.	- .20±6.6	4.66±7.2	204.	24.66±6.0	29.62±6.7
211.	10.04±3.1	9.03±5.9	212.	19.79±6.4	18.78±8.1	214.	29.81±4.4	28.80±6.7
221.	27.78±4.8	20.04±6.6	222.	38.69±4.7	30.95±6.6	224.	42.88±5.4	35.14±7.1
241.	33.20±6.0	-4.86±8.1	242.	40.71±6.7	2.65±8.6	244.	52.05±5.2	13.99±7.5
Mean.	16.44±2.2	5.96±3.2	Mean.	24.75±3.1	14.26±3.8	Mean.	37.35±2.6	26.86±3.5
401.	16.63±4.4	18.21±5.1	402.	6.26±4.5	7.84±5.2	404.	-2.37±4.7	- .79±5.4
411.	18.05±4.2	19.60±8.9	412.	19.45±5.1	21.00±9.3	414.	29.89±6.0	31.44±9.8
421.	13.65±6.2	-17.91±8.7	422.	35.15±4.6	3.59±7.6	424.	40.80±4.6	9.24±7.6
441.	34.85±5.9	8.53±7.7	442.	21.74±2.6	-4.58±5.5	444.	19.01±3.5	-7.31±6.0
Mean.	20.80±2.6	7.11±3.9	Mean.	20.65±2.1	6.96±3.5	Mean.	21.83±2.4	8.14±3.7

The striking feature about the curves in Figures 5 and 6 is the persistent recurrence of three ratios of nitrogen and potash, 2:4, 1:2, and 4:1. Expressing the ratios in terms of N and K₂O per acre they would be 15-0-100, 7.5-0-50, and 30-0-25. The first two ratios, 15-0-100 and 7.5-0-50, merely differ as to quantity.

In nitrogen-potash mixtures where phosphorus is omitted, the efficiency of potash increases in relation to successive increments of nitrogen in but a single series, namely, 001-101-201-401, as shown in Figure 5, A, for edible ears and in Figure 5, C, for fodder. In this series, potash is constant with a single dosage. The mean factors which include phosphorus, when compared in the equivalent series, 0X1-1X1-2X1-4X1, show that 4X1 is the mode (fig. 5, B and D) for both ears and fodder, but the efficiency factors are both very small and not statistically significant. In the remaining curves of Figure 5, A and C, treatments 102 or 204 are the modes where there are any. Adding phosphorus as in Figure 5, B and D, does not change the modes except in one series, 0X4-1X4-2X4-4X4, where 0X4 is the highest treatment.

Most of the efficiency factors considered in Figure 5 lack statistical significance. However, the curves are consistent in their trends to an appreciable degree.

Considering next the effect of increasing amounts of potash upon its own efficiency, nitrogen being held constant, the curves in Figure 6, A and C, where phosphorus is omitted, show that the efficiency of potash increases when the double dosage of nitrogen is held constant and decreases when the quadruple nitrogen dosage is held constant. Treatments 102, 204, or 401 are the modes of the six curves, although but three of these, according to Table 4 are significant.

Where phosphorus is included (fig. 6, B and D) potash efficiency increases just about as rapidly in relation to added increments without nitrogen as with this element included. As a matter of fact, in the case of edible ears the series 1X1-1X2-1X4 is the only one with nitrogen present that actually shows an increase higher than that where nitrogen is omitted. Fodder, on the other hand, gives increases in two series containing nitrogen, namely, 1X1-1X2-1X4 and 2X1-2X2-2X4.

Since the efficiency of potash is influenced to a considerable extent by nitrogen, it appears possible to draw certain general conclusions, with the reservation in mind, of course, that a considerable portion of the data have large probable errors. Figures 5 and 6 show the following:

With the single dosage of potash constant, the efficiency of potash tends to increase in relation to successive increments of nitrogen in mixtures containing no phosphorus (001-101-201-401).

With the double dosage of potash constant there is a considerable tendency for treatment 102 to be dominant where no phosphorus is used and 1X2 where phosphorus is included.

With the quadruple dosage of potash constant there is a tendency in the case of fodder for treatment 204 to give the maximum efficiency in mixtures omitting phosphorus and 2X4 where it is included.

Where successive increments of potash are added alone, there is a very slight tendency for the efficiency to increase in relation to the dosage, but this is confined to edible ears alone and the factors are not statistically significant.

In nitrogen-potash mixtures, treatments 102, 204, and 401 are dominant in general where the single, double, and quadruple dosages of nitrogen are held constant and potash increases. The probable errors in some instances are very large, however.

Increasing potash in phosphorus-potash mixtures likewise increases the efficiency of potash.

Increasing potash where phosphorus is included and nitrogen held constant shows that treatments 1X2, 2X4, and 4X1 are, with certain qualifications, dominant.

EFFECT OF POTASH ON THE EFFICIENCY OF NITROGEN

The discussion of the action of potash upon the efficiency of nitrogen is based on the data in Table 5 and Figures 7 and 8. The curves in Figure 7, A and C, and Figure 8, A and C, show that in N-K mixtures omitting P, treatments 102, 204, and 401 are dominant. All three are statistically significant on the fodder basis, only 401 on the ear basis. In Figure 7, B and D, and Figure 8, B and D, the modes for the mean factors show that 1X2, 2X4, and 4X1 are dominant on the fodder basis, but 1X2 is the only significant treatment. On the ear basis the factors are small and the probable errors large, so that no use can be made of the data.

If the N-K mixtures are arranged so that N increases and K remains constant as in Figure 8, A and C, then 102, 204 and 401 are again dominant. With respect to the mean factors, 4X1, 1X2, and 1X4 are the dominant treatments on the fodder basis, according to Figure 8, D.

In determining the effect of nitrogen upon the efficiency of potash, and reciprocally the effect of potash upon the efficiency of nitrogen,

the constant recurrence of similar N-K ratios as modes in the curves is rather marked. The maximum efficiency of both nitrogen and potash seems to be attained in certain fairly well defined ratios; and while there is some variation in these tendencies, it will be observed that at the same time there are scarcely ever any substitutions for the 1:2, 2:4, and 4:1 ratios of N and K. In view of this behavior the following conclusions may be drawn: (1) Where small dosages of nitrogen such as 7.5 pounds per acre are used, the highest efficiency is attained in combination with a large dosage of potash such as 50 pounds K_2O per acre; (2) a doubled dosage of nitrogen (15 pounds per acre) apparently requires for the maximum efficiency double the amount of potash, that is, 100 pounds K_2O per acre; and (3) quadrupled dosages of nitrogen (30 pounds per acre), are almost always

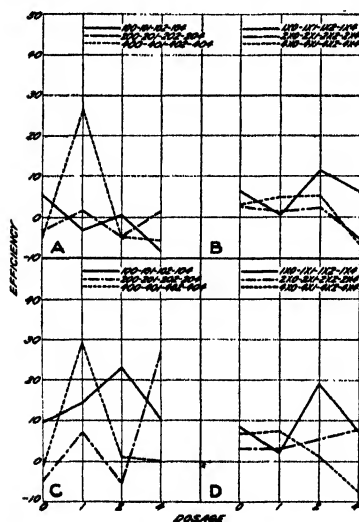


FIGURE 7.—The effect of successive increments of potash on the efficiency of nitrogen, both with and without phosphorus; results for two elements shown in A and C, for three elements in B and D, for edible ears in A and B, and for green fodder in C and D

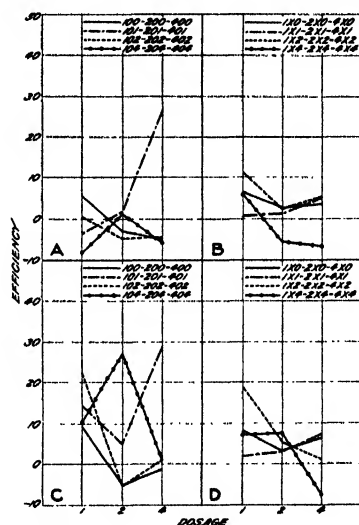


FIGURE 8.—The effect of successive increments of nitrogen on its efficiency in nitrogen-potash combinations, both with and without phosphorus; results for two elements shown in A and C, for three elements in B and D, for edible ears in A and B, and for green fodder in C and D

associated with the smallest potash dosage, that is, 25 pounds K_2O per acre.

The rather unusual feature is that potash affects nitrogen efficiency in practically the same manner as nitrogen affects potash efficiency. On this evidence alone, without supporting chemical data, one would assume that nitrogen can be used to replace potash, or that potash can be used to replace nitrogen.

This raises one of the most complex questions encountered in soils investigations—the question of the relations existing between nitrogen and potash and the determination of the optimum ratios between them. The trends shown by the curves in Figures 5, 6, 7, and 8 raise several interesting points essential to the successful solution of the problem. Briefly, the data in Figures 5 to 8 may be stated as

follows: (1) The 30-0-25 or 401 ratio is considerably higher in efficiency than are the 7.5-0-50 and the 15-0-100 (102 and 204) ratios on the basis of edible ears and tends to be equally efficient on the basis of green fodder; and (2) when phosphorus is included, the mean factors show that the 7.5-X-50 (1X2) ratio is usually considerably better than either the 15-X-100 (2X4) or the 30-X-25 (4X1) ratio as based on both edible ears and fodder.

TABLE 5.—*Comparison of the effect of potash on the mean percentage increases in yields of edible green sweet corn and of sweet-corn fodder, and on the efficiency of nitrogen, basis of percentages*

EDIBLE GREEN SWEET CORN

Treatment	Increase in yield	N efficiency factor	Treatment	Increase in yield	N efficiency factor	Treatment	Increase in yield	N efficiency factor
100....	5.51±5.0	5.51±5.0	200....	-2.91±2.2	-2.91±2.2	400....	-4.85±2.6	-4.85±2.6
110....	12.62±2.7	-10.01±5.5	210....	4.96±5.5	-17.67±7.3	410....	14.81±4.9	-7.82±6.8
120....	28.93±3.9	18.91±4.7	220....	31.60±4.8	21.48±5.5	420....	38.98±4.4	28.96±5.2
140....	30.12±6.1	10.67±7.3	240....	29.16±4.1	9.71±5.7	440....	15.73±4.0	-3.72±5.6
Mean..	19.30±2.3	6.27±2.8	Mean..	15.68±2.2	2.65±2.7	Mean..	16.17±2.0	3.14±2.6
101....	-12.41±6.5	-3.43±7.0	201....	-7.17±1.8	1.81±3.2	401....	17.38±3.3	26.36±4.3
111....	9.07±3.5	-4.84±5.3	211....	14.45±5.0	.54±6.4	411....	16.06±5.1	2.15±6.5
121....	34.47±4.7	15.72±6.4	221....	31.92±5.2	13.17±6.7	421....	22.35±7.0	3.60±8.2
141....	32.76±6.3	-4.65±7.9	241....	26.20±4.8	-11.21±6.8	441....	24.94±2.7	-12.47±5.5
Mean..	15.97±2.7	.70±3.4	Mean..	16.35±2.2	1.08±3.0	Mean..	20.18±2.4	4.91±3.2
102....	1.89±4.7	.26±4.9	202....	-2.91±4.8	-4.54±5.0	402....	-3.01±4.3	-4.64±4.6
112....	34.73±5.3	16.74±7.3	212....	31.95±6.3	13.96±8.0	412....	12.14±3.3	-5.85±6.0
122....	44.47±8.1	25.95±9.8	222....	29.93±5.2	11.41±6.9	422....	32.04±3.2	13.52±5.6
142....	40.11±5.8	2.83±7.3	242....	25.66±7.3	-11.62±8.6	442....	13.13±3.1	-24.15±5.5
Mean..	30.30±3.1	11.44±3.7	Mean..	21.16±3.0	2.30±3.6	Mean..	13.58±1.8	5.28±2.7
104....	-4.70±3.9	-8.28±5.0	204....	5.32±5.9	1.74±6.7	404....	-2.24±3.7	-5.82±4.9
114....	38.54±5.1	10.92±7.4	214....	25.10±5.2	-2.52±7.5	414....	18.12±7.0	2.50±8.8
124....	31.11±5.3	9.75±6.3	224....	16.50±5.9	-4.86±6.8	424....	35.53±2.5	14.17±4.2
144....	55.58±7.7	12.08±10.1	244....	27.27±3.1	-16.23±7.2	444....	5.89±3.1	-37.61±7.2
Mean..	30.13±2.8	6.12±3.7	Mean..	18.55±2.6	-5.47±3.5	Mean..	17.32±2.2	-6.69±3.3

GREEN SWEET-CORN FODDER

Treatment	Increase in yield	N efficiency factor	Treatment	Increase in yield	N efficiency factor	Treatment	Increase in yield	N efficiency factor
100....	9.74±8.1	9.74±8.1	200....	-4.86±3.0	-4.86±3.0	400....	-1.58±2.6	-1.58±2.6
110....	14.71±3.6	-.93±5.4	210....	1.01±5.0	-14.63±6.4	410....	-1.55±7.8	-17.19±8.8
120....	13.91±3.3	2.45±5.3	220....	7.74±4.6	-3.72±6.2	420....	31.56±6.1	20.10±7.4
140....	23.09±4.9	21.83±31.9	240....	38.06±5.4	36.80±32.0	440....	26.32±4.9	25.06±31.9
Mean..	15.36±2.7	8.27±8.4	Mean..	10.49±2.3	3.40±8.3	Mean..	13.69±2.8	6.60±8.5
101....	2.09±7.0	14.33±10.0	201....	-5.25±2.8	6.99±2.9	401....	16.63±4.4	28.87±4.4
111....	-1.77±3.6	-12.74±4.8	211....	10.04±3.1	-.93±4.9	411....	18.05±4.2	7.08±5.7
121....	26.66±5.7	3.48±7.0	221....	27.78±4.8	4.80±6.3	421....	13.66±6.2	-9.53±7.4
141....	35.46±5.7	3.17±7.7	241....	33.20±6.0	.91±7.9	441....	34.86±5.9	2.56±7.9
Mean..	15.61±2.8	2.06±3.8	Mean..	16.44±2.2	2.89±2.9	Mean..	20.80±2.6	7.25±3.2
102....	28.22±4.1	23.15±5.3	202....	-20.6±6.6	-5.27±7.4	402....	6.26±4.5	1.19±5.6
112....	42.34±5.3	28.76±6.9	212....	19.79±6.6	6.21±7.8	412....	19.45±5.1	5.87±6.7
122....	34.30±6.5	8.68±8.4	222....	38.69±4.7	13.07±7.1	422....	35.15±4.5	9.53±7.0
142....	48.45±4.4	15.43±7.2	242....	40.71±6.7	7.69±8.8	442....	21.74±2.6	-11.28±6.3
Mean..	38.33±2.6	19.00±3.5	Mean..	24.75±3.1	5.42±3.9	Mean..	20.65±2.1	1.33±3.2
104....	7.70±5.5	10.14±6.5	204....	24.66±6.0	27.10±6.9	404....	-2.37±4.7	.07±6.9
114....	35.91±3.8	-3.45±6.3	214....	20.81±4.4	-9.55±6.7	414....	29.89±6.0	-9.47±7.8
124....	36.24±6.4	2.50±8.1	224....	42.88±5.4	9.14±7.4	424....	40.80±4.6	7.06±6.8
144....	68.36±8.6	20.74±10.0	244....	52.05±5.2	4.43±7.4	444....	19.01±3.5	-28.61±6.3
Mean..	37.05±3.2	7.48±3.9	Mean..	37.35±2.6	7.78±3.6	Mean..	21.83±2.4	-7.74±3.4

From these results it appears that on soils of the type used here the fact that a ratio of nitrogen and potash such as the 30-0-25 gives the highest efficiency, might indicate that phosphorus is still to some extent a limiting factor. When the phosphorus requirement is satisfied, there should be a tendency toward greater efficiency in the direction of higher potassium and lower nitrogen.

THE EFFECT OF POTASH ON THE EFFICIENCY OF PHOSPHORUS

The effect of potash on the efficiency of phosphorus is given in Table 6, and Figures 9 and 10.

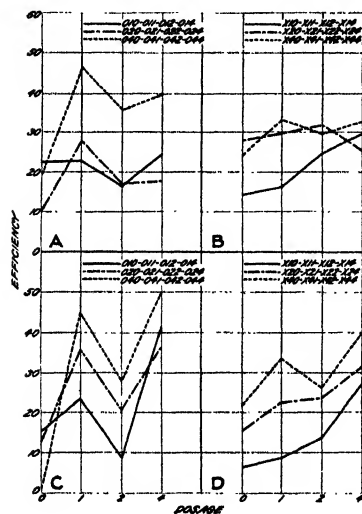


FIGURE 9.—The effect of successive increments of potash on the efficiency of phosphorus, both with and without nitrogen; results for two elements shown in A and C, for three elements in B and D, for edible ears in A and B, and for green fodder in C and D

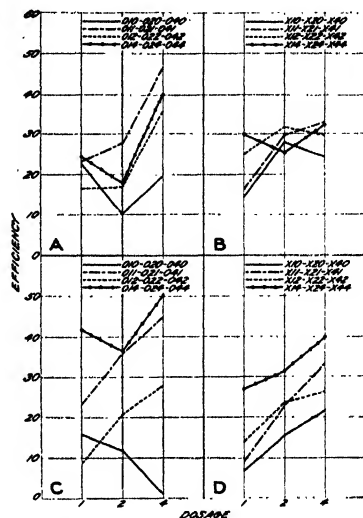


FIGURE 10.—The effect of successive increments of phosphorus on its efficiency in phosphorus-potash combinations, both with and without nitrogen; results for two elements shown in A and C, for three elements in B and D, for edible ears in A and B, and for green fodder in C and D

The curves in Figure 9, A, show that on the basis of edible ears, the efficiency of phosphorus does not tend to increase beyond the single dosage of potash. In one series, namely 010-011-012-014, potash seems to have but a very slight effect.

A somewhat different relationship is shown by the mean factors, where nitrogen is included, in Table 6 and Figure 9, B. The modes of the three curves are treatments X14, X22, and X41. In Figure 9, B, the curve for the series X40-X41-X42-X44 appears to be bimodal, treatment X44 being virtually the same as X41. The comparison of these four modes, namely, X14, X22, X41, and X44, indicates that although the treatments differ radically, the phosphorus efficiency factors are virtually the same.

In Figure 10, A and B, the effect of increasing dosages of phosphorus upon the efficiency of this element is shown on the basis of edible ears. Where nitrogen is omitted (fig. 10, A), phosphorus increases in efficiency in relation to the dosage, but not where it is used alone. Where nitrogen is included in Figure 10, B, the mean factors show

that treatments X20, X41, X22, X44, and X14 are the modes. In other words, the most efficient dosages are in one instance, low phosphorus; in two instances, double phosphorus; and in two, quadruple phosphorus, assuming curve X14-X24-X44 to be bimodal. From the data in Table 6 it appears that the differences between the efficiency factors for these treatments are very slight indeed.

TABLE 6.—*Comparison of the effect of potash on the mean percentage increases in yields of edible green sweet corn and of sweet-corn fodder, and on the efficiency of phosphorus, basis of percentages*

EDIBLE GREEN SWEET CORN

Treatment	Increase in yield	P efficiency factor	Treatment	Increase in yield	P efficiency factor	Treatment	Increase in yield	P efficiency factor
010.....	22.63±4.8	22.63±4.8	020....	10.02±2.7	10.02±2.7	040.....	19.45±4.0	19.45±4.0
110.....	12.62±2.7	7.11±5.7	120.....	28.93±3.9	23.42±6.3	140.....	30.12±6.1	24.61±7.9
210.....	4.96±5.5	7.87±5.9	220.....	31.50±4.8	34.41±5.3	240.....	29.16±4.1	32.07±4.6
410.....	14.81±4.9	19.66±5.5	420.....	38.98±4.4	43.83±5.1	440.....	15.73±4.0	20.58±4.8
Mean.....	13.76±2.3	14.32±2.7	Mean.....	27.36±2.0	27.92±2.5	Mean.....	23.62±2.3	24.18±2.8
011.....	13.91±4.0	22.89±4.8	021.....	18.75±4.3	27.73±5.1	041.....	37.41±4.8	46.39±5.5
111.....	9.07±3.5	21.48±7.4	121.....	34.47±4.7	46.88±6.8	141.....	32.76±6.3	45.17±9.0
211.....	14.45±5.0	21.62±5.3	221.....	31.92±5.2	39.09±5.5	241.....	26.20±4.8	33.37±5.1
411.....	10.06±5.1	-1.32±6.1	421.....	22.35±7.0	4.97±7.7	441.....	24.94±2.7	7.56±4.3
Mean.....	13.37±2.2	16.17±3.0	Mean.....	26.87±2.7	29.67±3.2	Mean.....	30.33±2.4	33.12±3.1
012.....	17.99±5.0	16.36±5.2	022.....	18.52±4.6	16.89±4.9	042.....	37.28±4.5	35.65±4.8
112.....	34.73±5.3	32.84±7.1	122.....	44.47±8.1	42.58±9.4	142.....	40.11±5.8	38.22±7.5
212.....	31.95±6.3	34.86±7.9	222.....	29.93±5.2	32.84±7.1	242.....	25.66±7.3	28.57±8.7
412.....	12.14±3.8	15.15±5.4	422.....	32.04±3.2	35.05±5.4	442.....	13.13±3.1	16.14±5.3
Mean.....	24.20±2.5	24.80±3.2	Mean.....	31.24±2.8	31.84±3.4	Mean.....	29.04±2.7	29.64±3.4
014.....	27.62±5.4	24.04±6.3	024.....	21.36±3.4	17.78±4.7	044.....	43.50±6.5	39.92±7.2
114.....	38.54±5.1	43.24±6.4	124.....	31.11±5.3	35.81±6.6	144.....	55.58±7.7	60.28±8.6
214.....	25.10±5.2	19.78±7.9	224.....	16.50±5.9	11.18±8.3	244.....	27.27±3.1	21.95±6.7
414.....	30.12±7.0	32.36±7.9	424.....	35.53±2.5	37.77±4.5	444.....	5.89±3.1	8.13±4.8
Mean.....	30.34±2.9	29.86±3.6	Mean.....	26.12±2.2	25.64±3.1	Mean.....	33.06±2.7	32.57±3.5

GREEN SWEET-CORN FODDER

010.....	15.64±4.0	15.64±4.0	020.....	11.46±4.1	11.46±4.1	040.....	1.26±31.5	1.26±31.5
110.....	14.71±3.6	4.97±8.9	120.....	13.91±3.3	4.17±8.7	140.....	23.09±4.9	13.35±9.5
210.....	1.01±5.0	5.87±5.8	220.....	7.74±4.6	12.60±5.5	240.....	38.06±5.4	42.92±6.2
410.....	-1.55±7.8	.03±8.2	420.....	31.56±6.0	33.14±6.6	440.....	26.32±4.9	27.90±5.5
Mean.....	7.45±2.7	6.63±3.5	Mean.....	16.17±2.3	15.34±3.2	Mean.....	22.18±8.2	21.36±8.5
011.....	10.97±3.8	23.21±8.1	021.....	23.18±4.0	35.42±8.2	041.....	32.20±5.2	44.53±8.9
111.....	-1.77±3.0	-3.86±7.6	121.....	26.66±5.7	24.57±9.0	141.....	35.46±5.7	33.37±9.0
211.....	10.04±3.1	15.29±4.2	221.....	27.78±4.8	33.03±5.6	241.....	33.20±6.0	38.45±6.6
411.....	18.05±4.2	1.02±6.1	421.....	13.65±6.2	-2.98±7.6	441.....	34.85±5.9	18.22±7.4
Mean.....	9.32±1.8	9.02±3.3	Mean.....	22.82±2.6	22.51±3.8	Mean.....	33.95±2.8	33.64±4.0
012.....	13.58±4.4	8.51±5.6	022.....	25.62±5.3	20.55±6.3	042.....	33.02±5.7	27.95±6.6
112.....	42.34±5.3	14.12±6.7	122.....	34.30±6.5	6.08±7.7	142.....	48.45±4.4	20.23±6.0
212.....	19.79±6.4	19.99±9.2	222.....	38.69±4.7	38.89±8.1	242.....	40.71±6.7	40.91±9.4
412.....	19.45±5.1	13.19±6.8	422.....	35.15±4.5	28.89±6.4	442.....	21.74±2.6	15.48±5.2
Mean.....	23.79±2.7	13.95±3.6	Mean.....	33.44±2.6	23.60±3.6	Mean.....	35.98±2.5	26.14±3.5
014.....	39.36±5.0	41.80±6.1	024.....	33.74±5.0	36.18±6.1	044.....	47.62±5.2	50.06±6.3
114.....	35.91±3.8	28.21±6.7	124.....	36.24±6.4	28.54±8.4	144.....	68.36±8.6	60.66±10.2
214.....	29.81±4.4	5.15±7.4	224.....	42.88±5.4	18.22±8.1	244.....	52.05±5.2	27.39±7.9
414.....	29.89±6.0	32.20±7.6	424.....	40.80±4.6	43.17±6.6	444.....	19.01±3.5	21.38±5.9
Mean.....	33.74±2.4	26.86±3.5	Mean.....	38.42±2.7	31.53±3.7	Mean.....	46.76±3.0	39.87±3.9

In Figure 9, A and B, the modes for the two sets of curves are as follows:

014, 021, and 041.
 X14, $\begin{cases} \text{X22 and X41.} \\ \text{X44.} \end{cases}$

Treatments X22 and X44 are bracketed together, as the ratios of P and K are identical. Treatment 021 is somewhat out of line in the comparisons made later. The relationship between phosphorus and potash, especially on the basis of the mean factors, is as follows:

P ₂ O ₅ -K	Pounds P ₂ O ₅	Pounds K ₂ O
1-4	32	100
2-2	64	50
4-1	128	25

The mean factors for these treatments are also quite similar, namely, 29.86 ± 3.6 for X14, 31.84 ± 3.4 for X22, and 33.12 ± 3.1 for X41. According to this, phosphorus and potash seem to have an inverse relationship that is more marked when nitrogen is included than when it is omitted.

The data showing the effect of potash on the efficiency of phosphorus as determined by green fodder (figs. 9, C, D, and 10, C, D) are derived from Table 6.

The curves in Figure 9, C, although consistently irregular, show that on the fodder basis the efficiency of phosphorus tends to increase in relation to the amount of potash applied, the heaviest potash treatment being the mode of each curve. The mean factors in Figure 9, D, show the same relationship, and the modes coincide with the highest potash dosage. This relationship, based on green fodder, is direct and comparatively simple when contrasted with the inverse relationship shown by the same series on the ear basis.

Increasing dosages of phosphorus tend to increase the efficiency of this element on the fodder basis under all conditions (fig. 10, C and D), except where phosphorus is applied alone in the series 010-020-040. The modes all contain quadruple phosphorus except treatment 010.

From the preceding discussion it appears that the efficiency of phosphorus is modified materially by potash both indirectly and directly. The tendencies mentioned are summarized in the following statements.

INDIRECT EFFECT OF POTASH ON THE EFFICIENCY OF PHOSPHORUS

On the basis of edible ears, but not on the fodder basis, increasing applications of potash tend to maintain an equivalent efficiency in relation to decreases in the amount of phosphorus used, this inverse relationship being limited to the mean factors in which nitrogen is included. (Fig. 9, B.)

In mixtures of phosphorus and potash, nitrogen omitted, the single dosage of potash is, in general, practically as efficient as heavier applications as determined by edible ears, or more so. (Fig. 9, A.)

DIRECT EFFECT OF POTASH ON THE EFFICIENCY OF PHOSPHORUS

On the green-fodder basis, the efficiency of phosphorus is found to increase directly in relation to the application of potash both in phosphorus-potash combinations and in those including nitrogen, namely, the mean factors. (Fig. 9, C and D.)

The efficiency of phosphorus when applied alone tends to decrease in relation to successive increases in the amount applied, such decreases being much more marked in the case of green fodder than in the case of edible ears. (Fig. 10, A and C.)

The efficiency of phosphorus tends to increase directly in relation to added increments of this element in phosphorus-potash mixtures containing no nitrogen, on the basis of both edible ears and fodder. (Fig. 10, A and C.)

Phosphorus shows the same tendency when nitrogen is included, as determined by the mean factors, except that a single discrepancy occurs in Figure 10, B, with reference to edible ears, treatment X22 being slightly higher than X42. (Fig. 10, B and D.)

EFFECT OF PHOSPHORUS ON THE EFFICIENCY OF POTASH

The data on the effect of phosphorus on the efficiency of potash are given in Table 7 and Figures 11 and 12. In view of the inverse relationship between phosphorus and potash, discussed under phosphorus efficiency, a similar relationship might be expected when potash efficiency is considered.

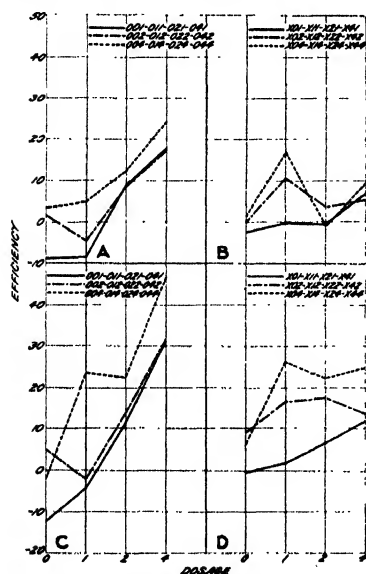


FIGURE 11.—The effect of successive increments of phosphorus on the efficiency of potash, both with and without nitrogen; results for two elements shown in A and C, for three elements in B and D, for edible ears in A and B, and for green fodder in C and D

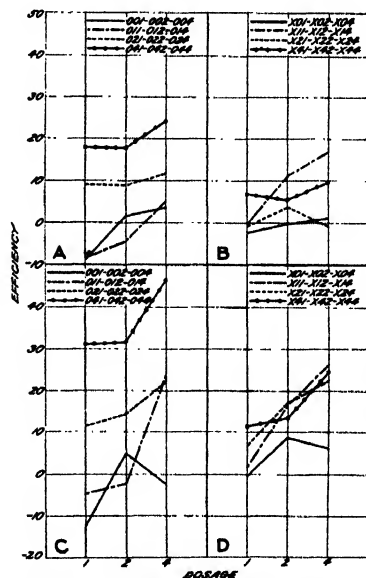


FIGURE 12.—The effect of successive increments of potash on its efficiency in phosphorus-potash combinations, both with and without nitrogen; results for two elements shown in A and C, for three elements in B and D, for edible ears in A and B, and for green fodder in C and D

According to Figure 11, A and C, the efficiency of potash based both on edible ears and on green fodder shows a tendency to increase in relation to successive additions of phosphorus when used in combinations of phosphorus and potash. As shown in the above tabulation, the mode of each curve coincides with the largest amount of

phosphorus used. The value of these factors as used in this interpretation must be qualified, of course, by the fact that the probable errors in most instances are large, and especially so in the case of fodder.

TABLE 7.—Comparison of the effects of phosphorus on the mean percentage increases in yields of edible green sweet corn and of sweet-corn fodder, and on the efficiency of potash, basis of percentages

EDIBLE GREEN SWEET CORN

Treatment	Increase in yield	K efficiency factor	Treatment	Increase in yield	K efficiency factor	Treatment	Increase in yield	K efficiency factor
001....	-8.98±2.7	-8.98±2.7	002....	1.63±1.6	1.63±1.6	004....	3.58±3.2	3.58±3.2
101....	-12.41±6.5	-17.92±8.2	102....	1.89±4.7	-3.62±6.9	104....	-4.70±3.9	-10.21±6.3
201....	-7.17±1.8	-4.26±2.8	202....	-2.91±4.8	0	204....	5.32±5.9	8.23±6.3
401....	17.38±3.3	22.23±4.2	402....	-3.01±4.3	1.84±5.0	404....	-2.24±3.7	2.61±4.5
Mean....	-2.80±2.0	-2.23±2.5	Mean....	-.60±2.0	-.04±3.1	Mean....	.49±2.2	1.05±2.6
011....	13.91±4.0	-8.72±6.2	012....	17.99±5.0	-4.04±6.9	014....	27.62±5.4	4.99±7.2
111....	9.07±3.5	-3.55±4.4	112....	34.73±5.3	22.11±5.9	114....	38.54±5.1	25.92±5.8
211....	14.45±5.0	9.49±7.4	212....	31.95±6.3	26.99±8.4	214....	25.10±5.2	20.14±7.6
411....	16.06±5.1	1.25±7.1	412....	12.14±3.3	-2.67±5.9	414....	30.12±7.0	15.31±8.5
Mean....	13.37±2.2	-.38±3.2	Mean....	24.20±2.5	10.45±3.4	Mean....	30.34±2.9	16.59±3.7
021....	18.75±4.3	8.73±5.1	022....	18.52±4.6	8.50±5.3	024....	21.36±3.4	11.34±4.3
121....	34.47±4.7	5.54±6.1	122....	44.47±8.1	15.54±9.0	124....	31.11±5.3	2.18±6.6
221....	31.92±5.2	42.7±1	222....	29.93±5.2	-1.57±7.1	224....	16.50±5.9	-15.00±7.6
421....	22.35±7.0	-16.63±8.3	422....	32.04±3.2	-6.94±5.4	424....	35.53±2.5	-3.45±5.1
Mean....	26.87±2.7	-.48±3.4	Mean....	31.24±2.8	3.88±3.4	Mean....	26.12±2.2	-1.23±3.0
041....	37.41±4.8	17.96±6.2	042....	37.28±4.5	17.83±6.0	044....	43.50±6.5	24.05±7.6
141....	32.76±6.8	2.64±8.8	142....	40.11±5.8	9.99±8.4	144....	55.58±7.7	25.46±9.8
241....	26.20±4.8	-2.96±6.3	242....	25.66±7.3	-3.50±8.4	244....	27.27±3.1	-1.89±5.1
441....	24.04±2.7	9.21±4.8	442....	13.13±3.1	-2.60±5.1	444....	6.89±3.1	-9.84±5.1
Mean....	30.33±2.4	6.71±3.3	Mean....	29.04±2.7	5.43±3.6	Mean....	33.06±2.7	9.44±3.6

GREEN SWEET-CORN FODDER

001....	-12.24±7.2	-12.24±7.2	002....	5.07±3.4	5.07±3.4	004....	-2.44±3.5	-2.44±3.5
101....	2.09±7.0	-7.65±10.7	102....	28.22±4.1	18.48±9.1	104....	7.70±5.5	-2.04±9.8
201....	-5.25±2.8	-.39±4.1	202....	-.20±6.6	4.06±7.2	204....	24.66±6.0	29.52±6.7
401....	16.63±4.4	18.21±5.1	402....	6.26±4.5	7.84±5.2	404....	-2.37±4.7	-.79±5.4
Mean....	.31±2.8	-.52±3.6	Mean....	9.84±2.4	9.01±3.3	Mean....	6.89±2.6	6.06±3.4
011....	10.97±3.8	-4.67±5.5	012....	13.58±4.4	-2.06±5.9	014....	39.36±5.0	23.72±6.4
111....	-1.77±3.0	-16.48±4.7	112....	42.34±5.3	27.63±6.4	114....	35.91±3.8	21.20±5.2
211....	10.04±3.1	9.03±5.9	212....	19.79±6.4	18.78±8.1	214....	29.81±4.4	28.80±6.7
411....	18.05±4.2	19.60±8.9	412....	19.45±5.1	21.00±9.3	414....	29.89±6.0	31.44±9.8
Mean....	9.32±1.8	1.87±3.2	Mean....	23.70±2.7	16.34±3.8	Mean....	33.74±2.4	26.29±3.6
021....	23.18±4.0	11.72±5.7	022....	25.62±5.8	14.16±6.8	024....	33.74±5.0	22.28±6.5
121....	26.66±5.7	12.75±6.6	122....	34.30±6.9	20.39±7.3	124....	36.24±6.4	22.53±7.2
221....	27.78±4.8	20.04±6.6	222....	38.69±4.7	30.95±6.6	224....	42.88±5.4	35.14±7.1
421....	13.65±6.2	-17.01±8.7	422....	35.15±4.6	3.59±7.6	424....	40.80±4.6	8.24±7.6
Mean....	22.82±2.6	6.65±3.5	Mean....	33.44±2.6	17.27±3.5	Mean....	38.42±2.7	22.25±3.6
041....	32.29±5.2	31.03±3.9	042....	33.02±5.7	31.76±32.0	044....	47.62±5.2	46.36±31.9
141....	35.46±5.7	12.37±7.5	142....	48.45±4.4	25.36±8.6	144....	68.36±8.6	45.27±9.9
241....	33.20±5.4	-4.86±8.1	242....	40.71±6.7	2.65±8.6	244....	52.05±5.2	13.90±7.5
441....	34.85±5.9	8.53±7.7	442....	21.74±2.6	-4.68±5.5	444....	19.01±3.5	-7.31±6.0
Mean....	33.95±2.8	11.77±3.5	Mean....	35.98±2.5	13.80±8.6	Mean....	46.76±3.0	24.58±8.7

According to Figure 12, A and C, there is more or less of a tendency for the efficiency of potash to increase in relation to the amount applied in phosphorus-potash mixtures based on both ears and fodder. When compared the modes of each curve confirm this, but the probable errors in several cases are very large indeed.

The curves of the mean factors, where nitrogen is included (fig. 11, B and D), show the influence of the inverse relationship between phosphorus and potash confirmed by the modes of each curve, namely, X41, X12, and X14 in the case of edible ears, and X41, X22, and X14 in the case of fodder. The mode X12 is out of line and should be X22. Four of these modes are more than three and two-tenths times their probable errors, one is more than three times, and another is twice its probable error.

The curves in Figure 12, B, consisting of the mean efficiency factors, show a tendency for the efficiency of potash to increase in relation to the amount applied. Only one of the modes, X14, is statistically significant, however. A similar tendency is indicated by fodder in Figure 12, D, but here the modes of each curve appear to be more significant.

In mixtures of phosphorus and potash, the tendency of potash to increase in efficiency is related (1) to successive increases in the amount of phosphorus applied, and (2) to successive increases in the amount of potash used. The mean factors when compared with respect to increasing dosages of phosphorus (fig. 11, B and D) show an inverse relationship, but when compared with respect to increasing dosages of potash (fig. 12, B and D), the maximum efficiency seems to be associated largely with the highest application of potash. From this it appears that while there is a tendency for phosphorus and potash to be inversely related on the basis of potash efficiency, such a tendency is by no means as strong or as consistent as the inverse relationship between these two elements based on phosphorus efficiency.

GENERAL DISCUSSION

The crop data have been interpreted by means of certain theoretical factors called efficiency factors. These factors show that each of the three nutrient elements essential to plant life is influenced by additions of salts containing one or two of the others. No attempt will be made to explain the causes of the phenomena observed. Whether they are due to changes in the availability of one fertilizer salt following the addition of one or two others is a matter of opinion that is outside the scope of this article. At the same time it should be understood that no claim is made concerning the probability that certain trends and tendencies observed in this experiment are applicable outside the limits of this experiment. The purpose of the discussion which follows is to correlate the apparently diverse trends of the efficiency factors in such a manner that the optimum fertilizer ratios of this experiment may be definitely determined.

The efficiency of nitrogen is influenced very materially by the presence of phosphorus and potash used in combination with it. Under the conditions of this experiment, nitrogen applied alone around the hill of corn has an efficiency which is slightly above or below zero. Preceding this top-dressing with a basal treatment of phosphorus gives variable results. If this basal treatment consists of 32 pounds P_2O_5 per acre the efficiency of nitrogen is depressed even further, but not to the extent of being statistically significant. Doubling the basal phosphorus treatment to 64 pounds P_2O_5 per acre gives very large increases in nitrogen efficiency, which in the case of edible ears are significant in all nitrogen combinations. Quadrupling the

phosphorus treatment to 128 pounds P_2O_5 per acre depresses the efficiency of nitrogen below the 64 pounds of P_2O_5 treatment in the case of edible ears but increases it in the case of fodder. However, none of the factors involving the basal treatment of 64 and 128 pounds of P_2O_5 as determined by fodder are significant. The mean factors which include the average of the potash additions show, with minor variations, substantially similar trends, with statistical significance almost entirely confined to the double phosphorus treatment, based on edible ears. The effect of increasing increments of nitrogen upon the efficiency of this element is somewhat variable. Significant results are obtained in only two instances, and both of these are based on edible ears. When combined with 64 pounds P_2O_5 per acre, nitrogen increases in efficiency as the dosage increases. The mean factors, however, show that the single nitrogen dosage is just as efficient as heavier applications. The remaining data also show that the single nitrogen dosage tends to be the most efficient.

Two points of major importance are brought out by this discussion. These are (1) that nitrogen, except where combined with an apparently optimum phosphorus dosage, tends to give the maximum efficiency when applied in quantities of not more than 7.5 pounds per acre, and (2) in order to obtain the highest efficiency, the nitrogen dosage must be combined with the double phosphorous dosage. This establishes the limits in this experiment for the use of nitrogen as determined by its relationship with phosphorus. It would be very difficult, indeed, to determine these facts with certainty from even a thorough study of the yields in Table 1.

The efficiency of nitrogen is also strongly influenced by the presence of a potash salt. The data show that this influence is reciprocal; in other words, the efficiency of potash seems to be affected by nitrogen in substantially the same manner as the efficiency of nitrogen is affected by potash. For this reason, the reciprocal efficiency of both of these elements will be discussed together. Although not entirely free from discrepancies, and qualified by the fact that the probable errors are very large, the curves in many instances show that the maximum efficiencies of both nitrogen and potash are associated in the following combinations: (1) $7\frac{1}{2}$ pounds nitrogen per acre side dressed after a basal treatment of 50 pounds K_2O per acre; (2) 15 pounds of nitrogen per acre side dressed after a basal treatment of 100 pounds K_2O per acre; and (3) 30 pounds of nitrogen per acre side dressed after a basal treatment of 25 pounds K_2O per acre.

Most of the factors for nitrogen efficiency as affected by potash and, inversely, potash efficiency as affected by nitrogen, are unreliable because of their large probable errors, but it should be noted that the statistically significant ones are practically all confined to the three ratios mentioned. Of these three, namely, 7.5-0-50, 15-0-100, and 30-0-25, the first two are the same except as to quantity. The last, 30-0-25, conforms approximately with commercial analyses such as 2-12-2, 4-16-4, etc., commonly used in Illinois and in other parts of the Central West, but the others are not in extensive commercial use. The data which have been presented show (1) that in combinations of nitrogen and potash, with phosphorus omitted with the 30-0-25 ratio is considerably more efficient than the other two ratios on the basis of edible ears and about equally so on the green-fodder basis; (2) that the mean factors which include phosphorus prove the 7.5-0-50 ratio

to be superior on the basis of both ears and fodder. It is therefore apparent that only two ratios of nitrogen and potash need be considered, a 30-0-25 and a 7.5-0-50, the latter being apparently superior to the former in complete analyses.

The discussion thus far has definitely limited the types of fertilizer analyses which might be expected to give optimum results. The requirements for an optimum analysis may be stated as follows: (1) Not more than 7.5 pounds of nitrogen per acre should be applied as a side dressing; (2) when nitrogen is used, this element gives the highest efficiency if combined with the double phosphorus application of 64 pounds P_2O_5 per acre; and (3) the ratio of nitrogen and potash that gives the best results in a phosphorus-bearing fertilizer is 7.5:50.

A fertilizer answering the above requirements would be a 7.5-64-50 in terms of plant food per acre. Before concluding that such a combination will fully answer the requirements of the soils in this experiment, a consideration of the efficiency of phosphorus is necessary.

The efficiency of phosphorus is influenced appreciably by the amount applied to the soil and also by the use of nitrogen in connection with it. Nitrogen acts in general as a depressive agent when side dressed after an application of 32 pounds P_2O_5 per acre; but when the application is increased to 64 or 128 pounds per acre, side dressings of 7.5 to 15 pounds nitrogen per acre are quite consistent in increasing the efficiency of phosphorus. These trends apply to nitrogen-phosphorus combinations. The mean efficiency factors have somewhat different trends. Based on edible ears, the single dosage of nitrogen is the most efficient, but as determined by green fodder, nitrogen has but little effect.

The efficiency of phosphorus changes materially in relation to the amount applied. Phosphorus alone tends to decrease in efficiency as successively larger amounts are applied. In nitrogen-phosphorus mixtures the tendency for phosphorus to increase in efficiency in relation to successive additions is quite marked. In general, 64 pounds P_2O_5 per acre is the most efficient on the edible-ear basis and from 64 to 128 pounds on the fodder basis. Although differing in some respects, the mean factors show substantially the same trends.

Since the efficiency factors just discussed are in most instances significant from the statistical standpoint, certain definite information is added concerning the optimum fertilizers in these experiments. It is obvious that nitrogen should not be used unless the phosphorus requirements are satisfied. Other important points brought out are (1) 7.5 pounds of nitrogen per acre is sufficient if it supplements both minerals, (2) 64 pounds P_2O_5 per acre may be considered the optimum phosphorus application, and (3) 32 pounds P_2O_5 per acre is the maximum application if phosphorus is to be used alone.

These trends justify the conclusion that 7.5 pounds N and 64 pounds P_2O_5 per acre is the combination that gives the optimum results. This is fully in accordance with the previous conclusion, that a 7.5-64-50 analysis on an acre basis gives the optimum results.

The efficiency of phosphorus is likewise modified to a considerable degree by potash, but green fodder and edible ears are not alike in their reaction. Considering the results with edible ears first, it has been found that on this basis there is a decided tendency for phosphorus and potash to bear an inverse relationship to each other, in which the mean efficiency factors tend to maintain an equilibrium as

the potash applications are increased and the phosphorus applications are decreased. (Table 8.) The mean factors are practically identical in size. In contrast, the efficiency of the single dosage of potash in mixtures of phosphorus and potash, omitting nitrogen, is as high as, or higher than, heavier applications, irrespective of the dosage of phosphorus.

TABLE 8.—*Inverse relationship between applications of phosphorus and potash as exemplified by equilibrium of the mean efficiency factors as potash applications are decreased and phosphorus applications increased*

[Figures relate to edible green sweet corn only]

Code	P ₂ O ₅	K ₂ O	Efficiency factor
	<i>Pounds per acre</i>	<i>Pounds per acre</i>	
X14.....	32	100	29.86±3.6
X22.....	64	50	31.84±3.4
X41.....	128	25	33.12±3.1

On the basis of green fodder, it is found that the efficiency of phosphorus increases directly in relation to the application of potash in all types of mixtures.

In phosphorus-potash mixtures containing no nitrogen, the efficiency of phosphorus increases directly in relation to the amount of phosphorus used. In mixtures including nitrogen, the general tendency is similar, but there are some important variations.

These results are of considerable value in determining the optimum phosphorus-potash ratio. The results show that mixtures of the minerals only respond differently from complete-analysis fertilizers. In mixtures of the minerals only, the highest efficiency is obtained with the single potash dosage on the ear basis, but higher applications of potash are indicated on the fodder basis. Similarly, the efficiency of phosphorus increases in relation to the dosage on both bases. Thus when phosphorus-potash mixtures are used the 041 type of mixture would give the highest efficiency on the ear basis, and the 044 type on the fodder basis. Stated in terms of plant-food ratios, the 041 mixture is equivalent to 0-128-125 and the 044 to 0-128-100. The nearest commercial analyses now available would be, respectively, 0-16-3 and 0-16-12.

However, this is materially modified by the mean factors where nitrogen is also averaged. Here equivalent efficiencies on the ear basis are shown by three radically different mixtures, X14, X22, and X41, which are equivalent to mixtures of X-32-100, X-64-50, and X-128-25; or, in trade terms, X-8-24, X-16-12, and X-16-3, applied, of course, in different quantities, 400, 400, and 800 pounds, respectively. On the fodder basis, a mixture of the X44 (X-128-100) type might be preferred, as the efficiency of phosphorus reaches the maximum when the highest dosages are used in connection with the highest dosages of potash.

The question now remains as to how these data may be properly evaluated. Previous to the discussion of the effect of potash on the efficiency of phosphorus, the conclusion was reached that a 7.5-64-50

per acre analysis would give the optimum results. A summary of the types of mixtures that give the best results is presented in Table 9.

TABLE 9.—Summary of types of fertilizer mixtures that give the best results with ear corn and fodder

Basis and basal formula	Nearest commercial formula	Quantity per acre	Plant food per acre (N-P ₂ O ₅ -K ₂ O)
Ear basis:		Pounds	Pounds
0-5-1-1	0-16-3	800	0-128-25
Fodder basis:			
0-1.3-1	0-16-12	800	0-128-100
Ear basis:			
X-1-3-1	X-8-24	400	X-32-100
X-1.3-1	X-16-12	400	X-64-50
X-5.1-1	X-16-3	800	X-128-25
Fodder basis:			
X-1.3-1	X-16-12	800	X-128-100

Considering first the fertilizers containing nitrogen, the X-128-25 type might be eliminated because, in order to obtain an equivalent efficiency, 800 pounds must be used, as compared with half the quantity in certain other mixtures. The X-32-100 mixture may also be eliminated because the efficiency of 7.5 pounds of nitrogen per acre, combined with 32 pounds P₂O₅ per acre is very low, and likewise combinations of 7.5 pounds nitrogen and 100 pounds K₂O per acre are very much less efficient than those containing half as much potash. This leaves the X-64-50 combination, which apparently fulfills all the requirements and is consistent with the 7.5-64-50 type already mentioned as being the most desirable.

Another type of mixture, because of its extensive use in Illinois, also requires discussion, that is, one containing only the minerals. Table 9 shows that the 0-128-25 is most efficient on the ear basis and the 0-128-100 on the fodder basis. The phosphorus efficiency factors of these mixtures on the fodder basis are, respectively, 44.53 ± 8.9 and 50.06 ± 6.3 . (Table 6.) The difference is 5.53 ± 10.9 , which is less than its probable error, and accordingly the 0-128-100 mixture may be eliminated. There is, however, one fundamental objection to the remaining 0-128-25 combination; it is an application much heavier than is customarily used in the Corn Belt for a low-value crop such as sweet corn. It is at this point that the experiment is weak, because there is no direct method of determining what the effect would be of halving the 0-128-25 treatment. The only alternative is to approximate the effect by indirect means. The treatments nearest to 0-128-25 are 0-128-0 and 0-128-50 (040 and 042). According to Table 6, the phosphorus efficiency of treatment 0-128-0 is only 19.45 ± 4.0 , whereas that of 0-128-50 is 35.65 ± 4.8 , or almost twice as large. If the respective half-size treatments 0-64-0 (020) and 0-64-25 (021) are compared (Table 6), the efficiencies are found to be 10.02 ± 2.7 and 27.73 ± 5.1 . Clearly, potash, because it gives such a large increase in phosphorus efficiency, is essential, and 0-128-0 and 0-64-0 can not be considered as alternative treatments in place of 0-128-25. This leaves 0-64-25 (021), which in turn is considerably higher than 0-64-50 (022) in phosphorus

efficiency. Therefore, the only possible treatment smaller than 0-128-50 (041) which approaches it in efficiency is 0-64-25 (021). A comparison of these treatments based on the data in Table 6 is shown in Table 10.

TABLE 10.—A comparison of two fertilizer treatments as to percentage increase in yield of ear corn and fodder and as to phosphorus efficiency factor

Treatment	Percentage increase in yield of—		Phosphorus-efficiency factor for—	
	Ears	Fodder	Ears	Fodder
0-128-50 (041).....	37.41±4.8	32.29±5.2	46.39±5.5	44.53± 8.9
0-64-25 (021).....	18.75±4.3	23.18±4.0	27.73±5.1	35.42± 8.2
Difference.....	18.66±6.4	9.11±6.6	18.66±7.5	9.11±12.1

All the differences recorded are less than three and two-tenths times their probable errors, and while the yield increase of 041 is double that of 021 the difference is somewhat less than might be regarded as significant. Numerous subsequent experiments in many sections of Illinois have proved the correctness of the belief that the 021 (0-64-25) mixture is the more desirable. It is the only mixture now in commercial use on thousands of acres of sweet corn in Illinois. It is applied at the rate of 100 pounds per acre around the hill at planting in the form of an 0-16-6 analysis that is one-fourth of the 0-64-25. Broadcasting has never become popular.

Consideration remains to be given to the effect of phosphorus on the efficiency of potash. It may be said in general that phosphorus exerts a weaker and less consistent effect upon potash than nitrogen. Most of the recorded efficiency factors lack statistical significance, but certain trends develop which have some value. In mixtures of phosphorus and potash there is a tendency for potash to increase in efficiency with successive increments of both phosphorus and potash. The mean efficiency factors show an inverse relationship similar to that discussed under phosphorus efficiency, but this tendency is comparatively limited in scope and is not so consistent.

The repeated process of elimination has brought the possible optimum fertilizer treatments in this experiment down to three, namely, 0-64-25, 0-128-25, and 7.5-64-50. Their relation to the yield data may now be determined by studying the crop records of edible ears from the 10 best treatments in Table 1, as shown in Table 11.

Two of the three treatments selected as being the optimum appear in the table. Treatment 122 (7.5-5-64-50) is third in rank on the basis of increased ton yields, but second on the percentage basis. Treatment 041 (0-128-25) is sixth on the basis of increased ton yields and seventh on the percentage basis. Treatment 021 (0-64-25) does not appear at all, but its multiple 042 (0-128-50) ranks about the same as 041.

TABLE 11.—*Relation between the efficiency of the three optimum fertilizer treatments as determined from crop records of edible ears produced by the 10 best treatments recorded in Table 1*

Treatment	Relationship computed—					
	On basis of yields by weight			On basis of percentage increase in yield		
	Rank	Tons per acre increase over check	Student's odds	Treatment	Rank	Percentage increase over check
144.....	1	0.971±0.134	3,332:1	144	1	55.58±7.7
142.....	2	.797±.115	1,932:1	122	2	44.47±8.1
044.....	3	.776±.115	1,799:1	044	3	43.50±6.5
122.....	4	.776±.142	400:1	142	4	40.11±5.8
420.....	4	.736±.083	>9,999:1	420	5	38.98±4.4
042.....	5	.683±.082	>9,999:1	114	6	38.54±5.1
041.....	6	.673±.086	4,999:1	041	7	37.41±4.8
141.....	7	.651±.125	323:1	042	8	37.28±4.5
424.....	7	.651±.046	>9,999:1	424	9	35.53±2.5
240.....	8	.648±.091	1,409:1	112	10	34.73±5.2

It is obvious that treatment 7.5-64-50 is much superior to the other two on the yield basis, and it should be noted that as compared with other high ranking ratios it contains the smallest amount of plant food. Four hundred pounds per acre of a 0-16-12 fertilizer followed by a side dressing of 50 pounds of sodium nitrate per acre would be the equivalent commercial application, and as a result of this work this is the one especially recommended.

SUMMARY

By means of a critical analysis of certain theoretical factors called efficiency factors, calculated from experimental results represented by six years' crop yields from 389 separate plots, the optimum fertilizer treatments are determined from a mass of apparently irrelevant and inconsistent crop yields without resorting to supplementary tests. This analysis is essentially a study of the reciprocal effects of fertilizer salts upon each other as determined by crop yields and not by chemical analysis. The influence of fertilizer salts upon each other was found to follow certain definite trends, which may or may not have a wider application than the limits of this experiment. The reciprocal action of these salts is given.

Nitrogen has a tendency to depress the efficiency of phosphorus when the latter element is applied in apparently insufficient amounts as a part of various fertilizer combinations; but if adequate amounts of phosphorus are included, small supplementary treatments of nitrogen increase the efficiency of phosphorus. Phosphorus applied alone in increasing amounts shows successive decreases in efficiency, but when used in combination with nitrogen, or with both nitrogen and potash, there is a consistent increase in efficiency.

Phosphorus also influences the efficiency of nitrogen to a very considerable extent. When the phosphorus content in nitrogen-phosphorus mixtures and in complete fertilizers appears to be inadequate, the efficiency of nitrogen is depressed, but if it is adequate there is an appreciable increase in nitrogen efficiency. When applications of nitrogen are increased and phosphorus is held constant the smallest amounts of nitrogen are usually the most efficient.

A study of the relations between nitrogen and potash indicates that these two nutrients have a reciprocal effect upon each other, and fur-

thermore their relationship shows a tendency to be inverse, that is, under certain conditions high nitrogen and low potash have efficiency factors equivalent to those derived from low nitrogen and high potash. With the inclusion of phosphorus, however, a combination containing low nitrogen and high potash usually gives better results than one with high nitrogen and low potash.

Phosphorus, in addition to being materially affected by nitrogen, is also subject to considerable change due to potash. The effect of potash is somewhat complex and does not manifest itself in the same manner under all conditions. Considered in terms of the influence upon yields of edible ears, potash seems to be most efficient when applied in relatively small amounts in combinations with phosphorus, but omitting nitrogen. The addition of nitrogen, as well as potash, gives rise to an inverse relationship in which phosphorus seems to maintain its efficiency in equilibrium when the potash dosage increases inversely to that of phosphorus. Considered from the standpoint of plant growth as determined by weight of green fodder, the efficiency of phosphorus tends to increase directly in relation to successive additions of potash. In general, the efficiency of phosphorus in various mixtures tends to increase in relation to the amounts applied.

The efficiency of potash is influenced to some extent by phosphorus and tends to increase in mixtures of these two elements (nitrogen omitted) according to the amount of each applied. There is a slight tendency for potash in complete fertilizers to exhibit the same type of inverse relationship with phosphorus as that mentioned in the preceding paragraph.

From a study of the efficiency factors it has been found possible to determine the optimum fertilizer ratios in this experiment without having direct recourse to the crop yields. It is believed that this method of analysis will be a valuable aid to the soils investigator in studying his data.

LITERATURE CITED

- (1) ANONYMOUS. *
1908. THE PROBABLE ERROR OF A MEAN. By Student. *Biometrika* 6: 1-25, illus.
- (2) APPLEMAN, C. O.
1923. FORECASTING THE DATE AND DURATION OF THE BEST CANNING STAGE FOR SWEET CORN. *Md. Agr. Expt. Sta. Bul.* 254, p. [47]-56, illus.
- (3) ——— and EATON, S. V.
1921. EVALUATION OF CLIMATIC TEMPERATURE EFFICIENCY FOR THE RIPENING PROCESSES IN SWEET CORN. *Jour. Agr. Research* 20:795-805, illus.
- (4) CULPEPPER, C. W., and MAGOON, C. A.
1924. STUDIES UPON THE RELATIVE MERITS OF SWEET CORN VARIETIES FOR CANNING PURPOSES AND THE RELATION OF MATURITY OF CORN TO THE QUALITY OF THE CANNED PRODUCT. *Jour. Agr. Research* 28: 403-443, illus.
- (5) LOVE, H. H.
1924. A MODIFICATION OF STUDENT'S TABLE FOR USE IN INTERPRETING EXPERIMENTAL RESULTS. *Jour. Amer. Soc. Agron.* 16:68-73.
- (6) ——— and BRUNSON, A. M.
1924. STUDENT'S METHOD FOR INTERPRETING PAIRED EXPERIMENTS. *Jour. Amer. Soc. Agron.* 16:60-68.
- (7) MYERS, M. T.
1930. DETERMINING THE DATE OF SILKING IN EXPERIMENTS WITH CORN. *Jour. Amer. Soc. Agron.* 22:280-283.
- (8) SPILLMAN, W. J.
1921. A PLAN FOR THE CONDUCT OF FERTILIZER EXPERIMENTS. *Jour. Amer. Soc. Agron.* 13:304-310.

JOURNAL OF AGRICULTURAL RESEARCH

VOL. 45

WASHINGTON, D. C., DECEMBER 15, 1932

No. 12

EFFICACY OF DIFFERENT STRAINS OF *BRUCELLA ABORTUS* AS IMMUNIZING AGENTS AGAINST INFECTIOUS ABORTION¹

By W. E. COTTON

Superintendent, Experiment Station, Bureau of Animal Industry, United States Department of Agriculture

PURPOSE OF THE INVESTIGATION

Previous experiments² with a strain of bovine *Brucella abortus*,³ which through long cultivation on artificial media had become so nearly avirulent for guinea pigs that it would no longer cause lesions in them except when injected in very large doses, showed that it possessed considerable value in protecting these animals from developing lesions of abortion disease when they were subsequently injected with a virulent strain of *Br. abortus*. Huddleson⁴ showed that a similar avirulent culture not only increased the resistance of guinea pigs to *Br. abortus* but also seemed to induce some degree of immunity in cattle.

Numerous attempts made at the Experiment Station, Bethesda, Md., about 10 years ago, to infect cattle by natural means with the swine type of *Brucella abortus* failed. These failures suggested the possibility that this type might be used to advantage in preparing an effective vaccine that would be free from the danger of infecting the treated animals. This idea was evolved before the greater danger of the swine type to human health was generally realized. It was hoped that both the avirulent bovine strain and the swine strain could be used on pregnant as well as on nonpregnant cattle without infecting them. Arrangements therefore were made to try these two strains on cows and heifers both before and after breeding; and, for the sake of comparison, to include a group of animals vaccinated, before service, with a virulent bovine strain.

¹ Received for publication March 10, 1932; issued December, 1932. This investigation was begun under the direction of E. C. Schroeder, former superintendent of the Experiment Station, to whom credit is given for a large share of the planning of the experiments and help in carrying them out. His untimely death in January, 1925, occurred before the work was completed.

² SCHROEDER, E. C., and COTTON, W. E. RECENT BUREAU OF ANIMAL INDUSTRY EXPERIMENT STATION BOVINE INFECTIOUS ABORTION STUDIES. Jour. Amer. Vet. Med. Assoc. 66: 550-561. 1925.

³ This organism was formerly referred to as *Bacillus abortus* or *Bacterium abortus*.

⁴ HUDDLESON, I. F. STUDIES ON A NONVIRULENT LIVING CULTURE OF BACT. ABORTUS TOWARDS PROTECTIVE VACCINATION OF CATTLE AGAINST BOVINE INFECTIOUS ABORTION (BANG'S ABORTION DISEASE). Mich. Agr. Expt. Sta. Tech. Bul. 65, 36 p. 1924.

THE VACCINAL IMMUNIZATION OF ANIMALS AGAINST BACT. ABORTUS (BANG) INFECTION. U. S. Livestock Sanit. Assoc. Proc. (1925) 23: 210-215. 1926.

SOURCES OF VACCINES

The avirulent bovine strain used in these investigations was isolated from the milk of a cow in 1915 and was then virulent. For many months this strain lived on culture media in a single tube so completely sealed with paraffin that there was practically no loss of moisture. After this period it was kept alive by frequent transfer on culture media without resort to animal passage. At the time of the experiment it had been avirulent for guinea pigs for several years, so far as producing lesions was concerned, but the microorganisms could be recovered from the spleens of the guinea pigs, in some instances, two months after inoculation. Though of very low virulence, the strain still retained its agglutinability in full measure. Four and one-half years before the experiment was begun, a pregnant heifer, negative to the blood test for abortion, was injected intravenously with the growth from one agar slant of this strain suspended in 20 c c of physiological salt solution. Thirty-seven days later she aborted an 8-month fetus and reacted to the agglutination test for abortion with a titer of 1 to 1,600. Guinea pigs inoculated with material from her placenta developed characteristic lesions of abortion disease, but those inoculated with her colostrum failed to do so. The cow's blood, a year later, was negative to the agglutination test. Nine months after the injection of the heifer mentioned above, two pregnant cows given three feedings of the attenuated culture failed to become infected or to react to the agglutination test.

The swine strain used in the investigation was from a sow that had acquired abortion disease through natural exposure to infected swine. This strain had been kept alive in a guinea pig for a number of months. The organism was isolated from the guinea pig a few months before the beginning of the experiment and produced well-marked characteristic abortion-disease lesions of the swine type in guinea pigs.

The virulent bovine strain was isolated from an aborting cow about six months before it was used in the experiment. It produced well-marked abortion-disease lesions of the bovine type in guinea pigs.

TESTS OF THE THREE VACCINES WHEN USED BEFORE CONCEPTION

METHOD AND TIME OF VACCINATION

To test the efficacy of the three vaccines when used before conception, they were administered to three groups of cows and heifers negative to the agglutination test for infectious abortion, and a similar group, untreated, was reserved for control. Each of the vaccines was prepared from four days' growth on 11 agar slants suspended in approximately 140 c c of 0.85 per cent salt solution, and all were of about the same density.

Each of the cattle in the first three groups received subcutaneously 20 c c of vaccine, 10 c c in front of each shoulder. With the exception of cow 1155 in the second group, the animals were vaccinated December 8, 1926. There was little systemic reaction in the cattle after vaccination, and the reactions were not very marked nor did they persist for long.

TABLE 1.—*Dates of vaccination and breeding of experimental cattle vaccinated before conception*

GROUP 1.—VACCINATED WITH AVIRULENT BOVINE STRAIN

Animal No.	Date of vaccination	Date of breeding	Interval between vaccination and breeding
	1926	1927	Days
Cow:			
1227	Dec. 8	Apr. 8	121
1365	do	Mar. 17	99
Heifer:			
1341	do	May 18	161
1344	do	Apr. 19	132
1348	do	Apr. 8	121
1369	do	Mar. 29	111

GROUP 2.—VACCINATED WITH VIRULENT SWINE STRAIN

Cow:			
1209	Dec. 8	Mar. 18	100
1155 *	Dec. 13	Apr. 5	113
1370	Dec. 8	do	118
Heifer:			
1346	do	Mar. 28	110
1350	do	Mar. 16	98
1356	do	Apr. 19	132

GROUP 3.—VACCINATED WITH VIRULENT BOVINE STRAIN

Cow:			
1142	Dec. 8	Apr. 5	118
1302	do	Mar. 22	104
1366	do	Apr. 5	118
Heifer:			
1345	do	Mar. 28	110
1347	do	May 16	159

GROUP 4.—CONTROLS, NOT VACCINATED

Cow:			
1248		July 8	
1340		May 14	
Heifer:			
1322		Apr. 2	
1323		do	
1325		May 11	
1342		Apr. 15	
1355		Mar. 17	

* Cow 1155, which had been vaccinated on Dec. 13, 1926, too late in pregnancy to be used in another experiment for which she was intended, was allowed to produce her calf, again bred Apr. 5, 1927, and added to this group to replace an animal that proved to be pregnant.

The cattle were served on the dates given in Table 1. With the exception of control cow 1248, all the animals in the experiment conceived within a period of 63 days of one another.

To test the virulence of the three vaccines, each was injected subcutaneously into 12 guinea pigs, the doses varying from $\frac{1}{4}$ to $\frac{1}{2}$ c c for the swine strain and virulent bovine strain, and from $\frac{1}{4}$ to 2 c c for the avirulent bovine strain. All guinea pigs receiving the swine and virulent bovine strains developed lesions of abortion disease characteristic of the respective types. None of the guinea pigs that received the avirulent bovine strain developed lesions.

EXPOSURE OF ANIMALS TO BRUCELLA ABORTUS

The four groups of animals were given six exposures to *Brucella abortus* infection by ingestion at intervals of from 7 to 17 days within a period of 55 days. The first of these exposures was made August 2, 1927, at which time most of the animals were from three and one-half to four and one-half months pregnant. In addition to the ingestion exposures, two drops of the material used in making them were instilled into one eye of each animal on two of the dates, August 29 and September 9, on which these exposures were made.

It was intended to use the organs of aborted bovine fetuses for the exposures, but only one such fetus could be obtained for the last exposure. For the remaining five exposures, therefore, use was made of diseased organs of guinea pigs that had been inoculated with fetal and placental material from aborting cows and of recently isolated *Br. abortus* cultures. Cultures alone were used in making two of the exposures, and cultures and guinea-pig tissues in the remaining ones. Emulsions were made of the bovine fetal material and of the guinea-pig organs by grinding in a mortar. Enough 0.85 per cent salt solution was added to make the mass sufficiently fluid to pass easily through a syringe, after which it was strained through cheesecloth to remove coarser particles. The suspensions of *Br. abortus* were prepared by washing the growth from 6-day agar slants with 0.85 per cent salt solution.

The exposures were made by placing the infectious material on the root of the tongue by means of a syringe. When the tissue emulsions were given, the dose was 40 c c. When the culture was given, the dose was equivalent to the growth on one agar slant suspended in 40 c c of 0.85 per cent salt solution. When tissue emulsions and cultures were combined, *Br. abortus* was added at the rate of about half the growth on one agar slant per animal, the dose of the combined materials being 40 c c.

Six guinea pigs were injected with each lot of material to test its virulence. These animals, in all cases, showed marked lesions of abortion disease when they were autopsied two months later. Had there not been doubt, at the time the exposures were made, of the virulence of the material used, fewer exposures would have been given.

The results of the exposures of the animals in the different groups vaccinated before conception follow. Tests of placentae, uterine discharges, fetal organs, colostrum, and milk were made for *Br. abortus* by guinea-pig inoculations unless otherwise noted.

GROUP 1. CATTLE VACCINATED WITH AVIRULENT BOVINE STRAIN

Cow 1227. Aborted September 26, 1927, 55 days after first exposure and 171 days after service, or in the sixth month of pregnancy; *Br. abortus* isolated from fetal organs by cultural methods; placenta and fetal organs produced typical abortion-disease lesions in guinea pigs; milk on November 23, 1927, and milk and supramammary glands on March 30, 1928, the date on which cow was killed, negative for *Br. abortus*.

Cow 1365. Gave birth to premature calf, which lived about an hour, October 11, 1927, 208 days after service and 70 days after first exposure; uterine discharges infected; milk positive for *Br. abortus* October 22, but negative November 22, 1927, and January 11, 1928; organs of calf negative for *Br. abortus*.

Heifer 1341. Produced a full-term (287-day) calf February 29, 1928, 211 days after first exposure; afterbirth normal in appearance and negative for *Br. abortus*; colostrum found to be infected March 1.

Heifer 1344. Gave birth to calf January 17, 1928, 273 days after service and 168 days after first exposure; calf died, without taking nourishment, a few hours

after it was born; afterbirth of normal appearance, passed normally, and negative for *Br. abortus*; stomach fluid and organs of calf negative. Though the record of this cow is incomplete because no tests were made of her milk for *Br. abortus*, agglutination tests of her blood, shown in Table 3, strongly indicate that she did not become infected. The calf, though slightly premature, probably died because of exposure to cold.

Heifer 1348. Gave birth to calf December 11, 1927, 247 days after service and 131 days after first exposure; calf died without taking nourishment; portion of afterbirth passed the next day had characteristic abortion-disease lesions; both it and organs of calf positive for *Br. abortus*; milk infected on 19 occasions and not infected on 2 others, at intervals from February 4 to November 28, 1928.

Heifer 1369. Gave birth to apparently normal calf December 27, 1927, 273 days after service and 147 days after first exposure; afterbirth much diseased and infected with *Br. abortus*; milk negative on February 25 and 28, 1928, but slightly infected March 3.

GROUP 2. CATTLE VACCINATED WITH VIRULENT SWINE STRAIN

Cow 1209. Aborted November 18, 1927, 245 days after service and 108 days after first exposure; placenta, fetal organs, and colostrum infected with *Br. abortus*; milk infected January 11 and 28 and February 4, 25, and 28, 1928.

Cow 1155. It was intended to use this animal in the experiment in vaccination after conception, but she was vaccinated so late in pregnancy that she produced a calf January 5, 1927, before she was exposed. She therefore received somewhat different treatment than the remaining animals in the group, being vaccinated 23 days before parturition and 114 days, or nearly 4 months, before the next service. She produced apparently normal calf January 17, 1928, 287 days after service and 137 days after first exposure; afterbirth of normal appearance and negative for *Br. abortus*; milk also negative on eight different days from February 25 to September 19, 1928, inclusive.

Cow 1370. Gave birth to apparently normal calf January 12, 1928, 282 days after service and 163 days after first exposure; afterbirth of normal appearance and negative for *Br. abortus*; milk negative February 24 and 28 and March 3, 1928, but when cow was killed April 28, 1928, milk taken from her udder and an emulsion of her supramammary glands produced abortion-disease lesions of the swine type in guinea pigs.

Heifer 1346. Gave birth to apparently normal calf December 29, 1927, 276 days after service and 149 days after first exposure; afterbirth passed normally and lost; uterine discharges negative for *Br. abortus*; milk negative on seven different days from January 11 to April 28, 1928, as were also the supramammary glands when the cow was killed April 28, 1928.

Heifer 1350. Aborted October 19, 1927, 217 days after service and 78 days after first exposure; afterbirth retained; *Br. abortus* isolated from lungs, liver, spleen, and stomach fluid of fetus; afterbirth infected; milk negative for *Br. abortus* October 22, 1927, and January 11 and 28 and February 25, 1928.

Heifer 1356. Aborted November 1, 1927, 196 days after service and 91 days after first exposure; fetal organs and uterine discharges infected with *Br. abortus*, but milk negative for this microorganism February 25 and 28 and March 3, 1928.

GROUP 3. CATTLE VACCINATED WITH VIRULENT BOVINE STRAIN

Cow 1142. Gave birth to apparently normal calf January 11, 1928, 281 days after service and 162 days after first exposure; afterbirth passed normally, of normal appearance, and negative for *Br. abortus*, but milk found to be infected with this microorganism January 28, February 25 and 28, and March 3, 1928.

Cow 1362. Gave birth to apparently normal calf December 30, 1927, 283 days after service and 150 days after first exposure; afterbirth passed normally, of normal appearance, and negative for *Br. abortus*; milk also negative January 28, February 4 and 28, and March 3, 1928, but produced slight abortion-disease lesions in one of two guinea pigs inoculated February 25, 1928.

Cow 1366. Died of enteritis October 7, 1927, 66 days after first exposure; uterus contained a 6-month fetus; uterus and contents apparently normal; guinea pigs inoculated from fetal organs, placenta, udder, and supramammary glands all remained normal.

Heifer 1345. Gave birth to apparently normal calf December 31, 1927, 278 days after service and 151 days after first exposure; afterbirth passed normally, of normal appearance, and negative for *Br. abortus*; milk also negative January 11, February 28, March 3, and October 12, 1928.

Heifer 1347. Gave birth to apparently normal calf February 15, 1928, 275 days after service and 197 days after first exposure; afterbirth passed normally,

of normal appearance, and negative for *Br. abortus*; milk negative February 24 and March 3, 1928.

Cattle 1142, 1362, 1345, and 1347 were again served within the first and second quarters of 1928 and were exposed during the periods of gestation which followed, along with cattle in another experiment and their controls. The exposures were made on August 3 and 9, when the animals were in their fifth, fifth, third, and fourth months of gestation, respectively. The exposure was sufficiently severe to cause two of five controls to become infected, one of which aborted and the other gave birth to a weak calf on the two hundred and fifty-eighth day of her gestation period. All four of the vaccinated animals in group 3, though they had received no protective treatment after vaccination and exposure, produced apparently normal, full-term calves. Guinea pigs inoculated with portions of placenta and uterine discharges from three of the cows were negative for *Br. abortus* on autopsy. No inoculations were made from the placenta of cow 1345, since her blood was negative to the agglutination test and had been so for more than six months. The colostrum of cow 1142 contained *Br. abortus*, but that from cows 1347 and 1362 did not, as shown by guinea-pig inoculations. The infection of the udder of cow 1142 dated from either the exposure or vaccination of the previous year, probably the latter, since her blood reaction persisted in a titer of 1 to 800 or higher after vaccination.

GROUP 4. CONTROL CATTLE

Cow 1248. Aborted February 23, 1928, 230 days after service and 205 days after first exposure; afterbirth had characteristic lesions of abortion disease; stomach and intestinal fluids and organs of fetus and placenta infected with *Br. abortus*; milk infected February 24 and 28 and March 30; cow was killed March 30 and her supramammary glands found to be infected.

Cow 1340. Gave birth to apparently normal calf February 23, 1928, 285 days after service and 205 days after first exposure; afterbirth lost; uterine discharges all negative for *Br. abortus* as to lesions and agglutination test; milk negative February 28 and March 3.

Heifer 1322. Aborted December 4, 1927, 246 days after service and 124 days after first exposure; afterbirth showed typical lesions of abortion disease; innumerable colonies of *Br. abortus* isolated from lungs of fetus; other organs and stomach and intestinal fluids of fetus and afterbirth infected with *Br. abortus* as was also milk January 11 and 28, February 4, 25, and 28, and March 3, 1928.

Heifer 1323. Aborted December 9, 1927, 251 days after service and 129 days after first exposure; afterbirth showed characteristic abortion-disease lesions; afterbirth infected with *Br. abortus* as were also organs and stomach and intestinal contents of fetus; no milk tests were made.

Heifer 1325. Aborted November 10, 1927, 183 days after service and 100 days after first exposure; afterbirth lost but uterine discharges characteristic of *Br. abortus* infection, and this microorganism isolated by cultural methods from stomach fluid, spleen, and lungs of fetus; uterine discharges infected but milk not infected November 22, 1927, the only time that it was tested.

Heifer 1342. Aborted November 12, 1927, 211 days after service and 102 days after first exposure; organs and stomach fluid of fetus and placenta and colostrum infected with *Br. abortus*; milk also infected November 22, 1927, February 25 and 28, March 3, August 11 and 29, October 20, 22, 23, 25, and 31, November 2, 7, 10, 15, and 20, 1928, producing marked lesions in all guinea pigs inoculated.

Heifer 1355. Gave birth to apparently normal calf December 19, 1927, 277 days after service and 139 days after first exposure; afterbirth passed normally, of normal appearance, and negative for *Br. abortus*; milk slightly infected February 25 but negative January 11 and 28, February 28, March 3, and April 12, 1928.

The results obtained in the tests with cows and heifers vaccinated before conception are presented in condensed form in Table 2. This

summary of data permits a ready comparison of the four groups. In the last two columns showing the relative resistance and percentage of full-term calves which lived, the reader will observe that both the percentage of resistance to infection and the percentage of living calves are much higher in the cattle vaccinated with the virulent bovine strain than in those vaccinated with the virulent swine or the avirulent bovine strains, and very much higher than in the controls. Furthermore, the percentage of udder infection is less in the cattle vaccinated with the virulent bovine and swine strains than in the controls. The significance of the data, however, is obviously somewhat limited because of the small number of cattle in the groups.

TABLE 2.—Comparative resistance to *Brucella abortus* exposure of cows and heifers vaccinated with different strains of *Br. abortus* before breeding and those not vaccinated

Group No.	Cattle	Strain of vaccine	Abortions or premature calves		Cows producing full-term calves		Cows producing infected or noninfected milk		Cows entirely resisting infection	Full-term calves which lived
			Caused by <i>Br. abortus</i>	Not caused by <i>Br. abortus</i>	After-birth infected	After-birth not infected	Infected	Noninfected		
	Number		Number	Number	Number	Number	Number	Number	Per cent	Per cent
1	6	Avirulent bovine	3	1	1	1	3	* 2	33.3	33.3
2	6	Virulent swine	3	0	0	3	* 2	4	33.3	50.0
3	4	Virulent bovine	0	0	0	4	2	2	50.0	100.0
4	7	None (control)	5	0	0	* 2	4	2	14.3	28.6

* No test was made of the milk of one of these animals, No. 1344, but agglutination tests of her blood and all other tests regarding her indicate that she entirely escaped infection.

† No. 1344 considered as being negative.

‡ Udder infection of 1 animal caused by vaccination and not by exposure.

§ Originally 5 animals; 1 died, leaving 4 in the group as sources of data.

|| Afterbirth of 1 animal lost but uterine discharges were negative for *Brucella abortus*

¶ Milk of 1 aborting animal was not tested.

EFFECTS OF VACCINATION AND EXPOSURE ON AGGLUTININ CONTENT OF BLOOD

Tables 3 to 6 give the results of agglutination tests made at intervals from the time of vaccination until after the termination of pregnancies. In general the tests were made in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, and 1:800. In a few instances the dilution was carried to 1:1,600 and 1:3,200. In some cases, when it seemed certain that the 1:25 dilution would not furnish additional data, it was omitted as were also the higher dilutions after the data obtained were deemed adequate.

The vaccine prepared from the avirulent bovine strain of *Br. abortus* (Table 3) induced a well-marked agglutination titer in the blood in 8 days. The titer was at its maximum at that time and had either entirely subsided or reached a low point 45 days later, except in one animal, in which it subsided more gradually.

The swine-strain vaccine (Table 4) induced the maximum agglutination titer in about 12 days and likewise soon began to subside, but did not so fully disappear as did the avirulent vaccine. The animal (No. 1370) in which the vaccine infected the udder retained a fairly high titer throughout the experiment.

The maximum titer induced by the virulent bovine-strain vaccine (Table 5) was reached by the sixteenth day and in two animals showed no tendency to subside, indicating that the vaccine had invaded the udder.

TABLE 3.—*Results of agglutination tests of blood sera of cattle in group 1 (vaccinated with an avirulent bovine strain of Brucella abortus before conception) when sera were in dilutions of 1:25, 1:50, 1:100, 1:400, 1:800, 1:1,600, and 1:3,200, respectively.*

Date of vaccination and first and last exposure	Date of agglutination test	Cow 1227	Cow 1365	Heifer 1341	Heifer 1344	Heifer 1348	Heifer 1369
Dec. 8, 1926, vaccinated	1926						
	Nov. 22	---	---	---	---	---	---
	Dec. 8	---	s---	s---	---	---	---
	Dec. 11	+++++	+++++	+++++	+++++	+++++	+++++
	Dec. 16	+++++	+++++	+++++	+++++	+++++	+++++
	Dec. 20	+++++	+++++	+++++	+++++	+++++	+++++
	1927						
	Jan. 4	+++++	+++++	+++++	+++++	+++++	+++++
	Jan. 12	+++++	+++++	+++++	+++++	+++++	+++++
	Feb. 5	+++++	+++++	+++++	+++++	+++++	+++++
	Feb. 17	+++++	+++++	+++++	+++++	+++++	+++++
	Feb. 26	+++++	+++++	+++++	+++++	+++++	+++++
	Mar. 14	+++++	+++++	+++++	+++++	+++++	+++++
Six exposures from Aug. 2 to Sept. 26, 1927, inclusive	Mar. 2	+++++	+++++	+++++	+++++	+++++	+++++
	Apr. 2	+++++	+++++	+++++	+++++	+++++	+++++
	May 10	+++++	+++++	+++++	+++++	+++++	+++++
	May 11	+++++	+++++	+++++	+++++	+++++	+++++
	July 1	+++++	+++++	+++++	+++++	+++++	+++++
	Aug. 3	+++++	+++++	+++++	+++++	+++++	+++++
	Aug. 9	+++++	+++++	+++++	+++++	+++++	+++++
	Aug. 18	+++++	+++++	+++++	+++++	+++++	+++++
	Aug. 30	+++++	+++++	+++++	+++++	+++++	+++++
	Sept. 13	+++++	+++++	+++++	+++++	+++++	+++++
	Sept. 23	+++++	+++++	+++++	+++++	+++++	+++++
	Sept. 26	+++++	+++++	+++++	+++++	+++++	+++++
	Oct. 5	+++++	+++++	+++++	+++++	+++++	+++++
1928	Oct. 12	+++++	+++++	+++++	+++++	+++++	+++++
	Nov. 3	+++++	+++++	+++++	+++++	+++++	+++++
	Dec. 5	+++++	+++++	+++++	+++++	+++++	+++++
	Dec. 12	+++++	+++++	+++++	+++++	+++++	+++++
	Dec. 23	+++++	+++++	+++++	+++++	+++++	+++++
1928	Jan. 11	+++++	+++++	+++++	+++++	+++++	+++++
	Mar. 1	+++++	+++++	+++++	+++++	+++++	+++++
	Mar. 3	+++++	+++++	+++++	+++++	+++++	+++++

• Key: + = complete agglutination; p = partial agglutination; s = slight agglutination; - = no agglutination; o = no test made in this dilution.
 • Sept. 26, aborted. • Oct. 16, aborted. • Dec. 11, calved prematurely. • Dec. 23, calved. • Jan. 17, calved prematurely. • Feb. 26, calved.

TABLE 4.—Results of agglutination tests of blood sera of cattle in group 2 (vaccinated with virulent swine strain of *Brucella abortus* before conception) when sera were in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600, and 1:3,200, respectively.

Date of vaccination and first and last exposure	Date of agglutination test	Cow 1209	Cow 1155 ^b	Cow 1370	Heifer 1346	Heifer 1350	Heifer 1356
Dec. 8, 1926, vaccinated	1926 Nov. 22	---	---	---	---	---	---
	Dec. 8	---	---	---	---	---	---
	Dec. 11	---	---	---	---	---	---
	Dec. 13	---	---	---	---	---	---
	Dec. 16	+++	+++	+++	+++	+++	+++
	Dec. 20	+++	+++	+++	+++	+++	+++
	1927 Jan. 4	+++	+++	+++	+++	+++	+++
	Jan. 22	+++	+++	+++	+++	+++	+++
	Feb. 5	+++	+++	+++	+++	+++	+++
	Feb. 17	+++	+++	+++	+++	+++	+++
	Feb. 23	+++	+++	+++	+++	+++	+++
	Mar. 14	+++	+++	+++	+++	+++	+++
	Apr. 12	+++	+++	+++	+++	+++	+++
	May 10	+++	+++	+++	+++	+++	+++
Six exposures from Aug. 2, to Sept. 26, 1927, inclusive	July 11	+++	+++	+++	+++	+++	+++
	Aug. 3	+++	+++	+++	+++	+++	+++
	Aug. 9	+++	+++	+++	+++	+++	+++
	Aug. 18	+++	+++	+++	+++	+++	+++
	Aug. 30	+++	+++	+++	+++	+++	+++
	Sept. 13	+++	+++	+++	+++	+++	+++
	Sept. 23	+++	+++	+++	+++	+++	+++
	Oct. 5	+++	+++	+++	+++	+++	+++
	Oct. 12	+++	+++	+++	+++	+++	+++
	Nov. 3	+++	+++	+++	+++	+++	+++
	Dec. 5	+++	+++	+++	+++	+++	+++
	Dec. 23	+++	+++	+++	+++	+++	+++
	Dec. 30	+++	+++	+++	+++	+++	+++
	1928 Jan. 11	+++	+++	+++	+++	+++	+++
	Jan. 17	+++	+++	+++	+++	+++	+++

* Key: + = complete agglutination; p = partial agglutination; s = slight agglutination; o = no agglutination; o = no test made in this dilution.

^b Cow 1155 vaccinated Dec. 13. * Jan. 5, calved. * Oct. 19, aborted. * Nov. 18, aborted. * Nov. 1, aborted. * Dec. 28, calved. * Jan. 12, calved. * Jan. 17, calved.

TABLE 5.—Results of agglutination tests of blood sera of cattle in group 3 (vaccinated with virulent bovine strain of *Brucella abortus* before conception) when sera were in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600, 1:3,200, respectively.*

Date of vaccination and first and last exposure	Date of agglutination test	Cow 1142	Cow 1362	Cow 1366	Heifer 1345	Heifer 1347
Dec. 8, 1924, vaccinated	1926 ^b					
	Nov. 22	-----	-----	s-----	-----	-----
	Dec. 8	s-----	s-----	s-----	-----	-----
	Dec. 11	-----	-----	s-----	-----	-----
	Dec. 16	o+++++s-	o+++++s-	p+ps	+++++s	+++++s
	Dec. 20	o+++++p	o+++++s	o+++++s	+++++s-	o+++++p-
	1927					
	Jan. 4	o+++++p	o+++++p	o+++++p-	o+ps-	o+++++p-
	Jan. 22	o+++++ps	o+++++p-	o+s-	o+++++s	o+++++p-
	Feb. 5	o+++++p	o+++++p-	o+s-	o+p-	o+s-
	Feb. 17	o+++++p	o+++++p-	o+s-	ops-	o+s-
	Feb. 23	o+++++p	o+++++s-	o+++++s-	ops-	o+s-
Six exposures from Aug. 2 to Sept. 26, 1927, inclusive	Mar. 14	o+++++s	o+++++s-	o+++++s-	ops-	o+s-
	Apr. 2	o+++++s	o+++++ps	o+++++s-	ops-	o+s-
	Apr. 10	o+++++p	o+++++ps	o+++++s-	ops-	o+s-
	May 11	o+++++p	o+++++ps	o+++++s-	ops-	o+s-
	July 3	o+++++p	o+++++ps	o+++++s-	ops-	o+s-
	Aug. 9	o+++++ps	o+++++ps	o+++++s-	ops-	o+s-
	Aug. 18	o+++++ps	o+++++ps	o+++++s-	ops-	o+s-
	Aug. 30	o+++++p	o+++++ps	o+++++s-	ops-	o+s-
	Sept. 13	o+++++p	o+++++ps	o+++++s-	ops-	o+s-
	Sept. 23	o+++++s	o+++++ps	o+++++s-	ops-	o+s-
	Oct. 5	o+++++s	o+++++ps	o+++++s-	ops-	o+s-
	Oct. 12	o+++++s	o+++++ps	o+++++s-	ops-	o+s-
1928	Nov. 3	o+++++s	o+++++ps	o+++++s-	ops-	o+s-
	Dec. 5	o+++++s	o+++++ps	o+++++s-	ops-	o+s-
	Dec. 23	o+++++p	o+++++ps	o+++++s-	ops-	o+s-
	Jan. 11	o+++++p	o+++++p-	o+++++p-	s-----	o+++++p-

* Key: + = complete agglutination; p = partial agglutination; s = slight agglutination; - = no agglutination; o = no test made in this dilution.

^b Oct. 7, died.

^c Dec. 30, calved.

^d Dec. 31, calved.

^e Jan. 11, calved.

^f Feb. 15, calved.

TABLE 6.—Results of agglutination tests of blood sera of cattle in group 4 (controls) when sera were in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600, 1:3,200, respectively^a

Date of first and last exposure	Date of agglutination test	Cow 1245	Cow 1340	Heifer 1322	Heifer 1323	Heifer 1325	Heifer 1342	Heifer 1355
Six exposures from Aug. 2, to Sept. 26, 1927, inclusive.	1926 Nov. 22	---	---	---	---	---	---	---
	1927 Feb. 17	0	0	0	---	0	0	0
	Mar. 14	0	---	---	---	s	---	---
	July 11	s	---	---	---	s	---	---
	Aug. 3	s	---	---	---	s	---	---
	Aug. 18	+	---	---	---	s	---	---
	Aug. 26	+	---	---	---	s	---	---
	Sept. 13	+	+	+	+	+	+	+
	Sept. 23	+	+	+	+	+	+	+
	Oct. 5	+	+	+	+	+	+	+
	Oct. 12	+	+	+	+	+	+	+
	Nov. 3	+	+	+	+	+	+	+
	Nov. 7	+	+	+	+	+	+	+
	Nov. 10	+	+	+	+	+	+	+
	Nov. 12	+	+	+	+	+	+	+
	Dec. 5	+	+	+	+	+	+	+
	Dec. 10	+	+	+	+	+	+	+
	Dec. 23	ojs	s	o	+	+	+	+
	1928 Jan. 11	ps	s	+	+	+	+	+
	Feb. 24	+	+	+	+	+	+	+
	Mar. 3	+	+	+	+	+	+	+

^a Key: + = complete agglutination; p = partial agglutination; s = slight agglutination; o = no test was made in this dilution.

^b Nov. 10, aborted. ^c Nov. 12, aborted. ^d Dec. 4, aborted. ^e Dec. 9, aborted. ^f Dec. 19, calved. ^g Feb. 23, calved.

TESTS OF TWO OF THE THREE VACCINES WHEN USED AFTER CONCEPTION

METHOD AND TIME OF VACCINATION

A second series of experiments was made to test the efficacy of the vaccines on pregnant cows and heifers. The avirulent bovine strain and the virulent swine strain previously described were used, but the virulent bovine strain was omitted because of the obvious likelihood that it would cause abortion in pregnant animals. The vaccines were prepared in the same manner as before and were of about the same density.

The animals in groups 1 and 2 were vaccinated on December 13, 1926, except one animal in the latter group, which was vaccinated on December 8. Each animal in group 1 received 20 c c of the avirulent bovine-strain vaccine subcutaneously, divided between two points, one in front of each shoulder. The total dose for each animal represented the growth on two well-grown agar slants. Each animal in group 2 received the same quantity of the virulent swine-type vaccine in the same manner, the dose in this case being equivalent to the growth on one and one-third agar slants. Twelve control guinea pigs that were injected subcutaneously with doses varying from $\frac{1}{4}$ to 2 c c of the avirulent bovine strain showed no abortion-disease lesions when killed. Six control guinea pigs, similarly injected, that received doses varying from one-sixteenth to one-fourth cubic centimeter of the swine-strain vaccine, developed well-marked and typical swine-abortion-disease lesions.

The service dates of only two of the animals used in the experiment were available. The remaining animals were examined for pregnancy a few days before the principals were vaccinated, and were estimated to have been pregnant for the period shown in Table 7.

TABLE 7.—*Dates of vaccination and breeding of pregnant experimental cattle, 1926*
GROUP 1.—VACCINATED WITH AVIRULENT BOVINE STRAIN *

Animal No.	Date of breeding *	Date of vaccination	Interval between vaccination and service *
			<i>Days</i>
Cow:			
1226.....	Sept. 14.....	Dec. 13.....	90
1361.....	Aug. 15.....	do.....	120
Heifer:			
1327.....	do.....	do.....	120
1367.....	July 16.....	do.....	150

GROUP 2.—VACCINATED WITH VIRULENT SWINE STRAIN

Cow: 1364.....	Aug. 30.....	Dec. 13.....	105
Heifer:			
1301.....	Sept. 14.....	do.....	90
1349.....	Aug. 15.....	do.....	120
1351.....	Sept. 14.....	Dec. 8.....	85
1358.....	Sept. 29.....	Dec. 13.....	75
1372 ^b	May 1.....	do.....	226

* Dates and intervals between vaccination and service for cows 1174 and 1338 are exact, those for the remaining animals are approximate.

^b This animal later was replaced by heifer 1351.

TABLE 7.—*Dates of vaccination and breeding of pregnant experimental cattle, 1926—Continued*

GROUP 3.—CONTROLS, NOT VACCINATED

Animal No.	Date of breeding	Date of vaccination	Interval between vaccination and service
			Days
Cow:			
1174.....	Sept. 10.....
1338.....	Oct. 13.....
Heifer:			
1300.....	Sept. 14.....
1352.....	Aug. 30.....
1357.....	Oct. 1.....
1371.....	Sept. 8.....

On December 14 and 15 some of the vaccinated cattle had elevated temperatures, but none were visibly sick. The local reactions were not very marked and subsided after a few days.

On January 5, 1927, cow 1155 of group 2 gave birth to an apparently normal calf; the afterbirth passed normally and was lost, but guinea pigs inoculated with uterine discharges remained normal. Milk on January 22, 1927, was negative for *Br. abortus*, by guinea-pig inoculations. On January 5, heifer 1343, of group 1, also gave birth to an apparently normal calf; the afterbirth was normal in appearance and negative for *Br. abortus*, by guinea-pig inoculations. Milk on January 22, 1927, also was negative for *Br. abortus*, by guinea-pig inoculations. Since both of these animals gave birth to calves before they were exposed, they were removed from this experiment.

EXPOSURE OF ANIMALS TO BRUCELLA ABORTUS

The animals in all three groups were given the same exposure, except that heifer 1351, which replaced heifer 1372, did not receive the first exposure, and heifers 1352 and 1371 were not given the last exposure, since their pregnancies terminated before it was made. The series of exposures extended over a period of 71 days.

In the first exposure, January 14, 1927, 5 c c of an emulsion of liver and stomach and intestinal contents of a bovine fetus, aborted as a result of natural infection, was placed on the root of the tongue of each animal in the three groups by means of a syringe with a piece of rubber tubing attached to its nozzle. *Brucella abortus* was isolated from the organs of the fetus, and 12 guinea pigs inoculated subcutaneously with from one-sixteenth to one-fourth cubic centimeter of the emulsion used in making the exposure developed typical bovine-abortion-disease lesions. The procedure was repeated on the following day, thus making the exposure of composite nature. Six guinea pigs inoculated with the emulsion on that day showed well-marked abortion-disease lesions at autopsy.

On February 5, 1927, heifer 1372 of group 2 gave birth to a normal calf; afterbirth was normal, and guinea pigs inoculated with it remained normal. As only 22 days had elapsed since exposure, this

heifer was replaced by heifer 1351. Heifer 1351 was vaccinated about five days earlier than the other animals in group 2, but otherwise received the same treatment.

In the second series of exposures, February 8, 9, and 10, 1927, each animal in the three groups was given, in the same manner as in the previous exposure, 10 c c of an emulsion of placenta of a cow that produced a premature calf which lived only about 24 hours. The placenta had the characteristic appearance of one infected with *Br. abortus*, and guinea pigs inoculated with the emulsion of it on each of the days it was given to the cattle developed abortion-disease lesions.

In the third series of exposures, February 23, 24, 25, 26, and 27, 1927, each animal of the three groups was given 20 c c of an emulsion of organs and stomach fluid of a bovine fetus, aborted as a result of natural exposure. Guinea pigs inoculated on each of these days with emulsion developed abortion-disease lesions.

In the fourth exposure, March 19, 1927, each animal in the three groups received 20 c c of an emulsion of organs and stomach fluid of a fetus from a cow that had been artificially infected to obtain material for exposure. In addition, two drops of the stomach fluid was placed in one eye of each of the cattle. Inoculation of guinea pigs proved the emulsion to be infectious but not markedly so.

In the fifth exposure, March 26, 1927, each animal in the three groups, except heifers 1352 and 1371 of group 3, whose pregnancies had terminated before the exposure was made, was given 20 c c of stomach fluid of the fetus from a cow that aborted on March 22, 1927. Guinea-pig inoculations showed that the material was thoroughly infectious.

The results of the exposures of the animals in the different groups vaccinated after conception follow. Tests of placentae, uterine discharges, fetal organs, colostrum, and milk were made for *Br. abortus* by guinea-pig inoculations unless otherwise noted.

GROUP 1. CATTLE VACCINATED WITH AVIRULENT BOVINE STRAIN

Cow 1226. Gave birth to normal calf June 27, 1927, 164 days after first exposure and 93 days after the last; afterbirth normal in appearance but infected with *Br. abortus*; milk, however, negative for this microorganism on July 11, 14, and 20 and October 6, 1927, and on January 12 and 28, February 25 and 28, and March 3, 1928.

Cow 1361. Aborted a small, 7½ to 8 months' fetus April 20, 1927, 96 days after first exposure and 25 days after the last; afterbirth not firmly retained but infected with *Br. abortus*; stomach fluid and organs of fetus not infected; milk not infected July 11, 14, and 20, and November 23, 1927, and January 12 and 28, and February 4, 1928; again became pregnant and on June 5, 1928, gave birth to apparently normal calf; afterbirth negative for *Br. abortus*.

Heifer 1327. Gave birth to apparently normal calf May 24, 1927, 130 days after first exposure and 59 days after the last; a few cotyledons of the placenta showed lesions of abortion disease and were found to be infected, as was also the milk July 11, 14, and 20, 1927. Cow was killed August 4, 1928; autopsy showed all organs to be apparently normal but supramammary glands proved to be infected with *Br. abortus*.

Heifer 1367. On April 5, 1927, udder developed, and at times from April 5 to 10 discharges from the vagina; on April 6 and 9 discharges used to inoculate guinea pigs but without infecting them. Gave birth to apparently normal calf April 25, 101 days after first exposure and 30 days after the last; afterbirth normal and proved to be negative for *Br. abortus*; milk negative for this microorganism July 11, 14, and 20 and November 23, 1927, and January 12 and 28, February 4, 25, and 28, and March 3, 1928. Gave birth to another apparently normal calf August 1, 1928; afterbirth negative for *Br. abortus*.

GROUP 2. CATTLE VACCINATED WITH VIRULENT SWINE STRAIN

Cow 1364. Gave birth to apparently normal calf June 10, 1927, 147 days after first exposure and 76 days after the last; afterbirth of normal appearance and 12 guinea pigs inoculated from it remained normal. Milk negative for *Br. abortus* July 11, 14, and 20, and November 23, 1927, and January 12 and 28, February 4, 25, and 28, March 3, May 8, 11, and 16, June 22, 26, and 29, and September 19, 1928.

Heifer 1301. Gave birth to apparently normal calf June 29, 1927, 156 days after first exposure and 85 days after the last; afterbirth apparently normal but 4 of 10 guinea pigs inoculated from it became infected. Milk negative for *Br. abortus* July 11, 14, and 20, and November 23, 1927, and January 12 and 28, February 4, 25, and 28, March 3, and May 8, 11, and 16, 1928.

Heifer 1349. Gave birth to apparently normal calf May 18, 1927, 124 days after first exposure and 53 days after the last; afterbirth appeared normal, and proved to be negative for *Br. abortus*; milk also negative July 11, 14, and 20, and November 23, 1927, and January 12 and 28, February 4, 25, and 28, March 3, May 8 and 11, June 22, 26, and 29, and September 19, 1928.

Heifer 1351. Gave birth to apparently full-term calf June 24, 1927, 136 days after first exposure and 90 days after the last; afterbirth apparently normal except for what seemed to be very small necrotic areas at the borders of several of the cotyledons. Ten guinea pigs inoculated with fragments of these cotyledons failed to develop lesions of abortion disease. Milk negative for *Br. abortus* July 11, 14, and 20, and November 23, 1927, and January 12 and 28, February 4, 25, and 28, March 3, May 8 and 11, June 22, 26, and 29, and September 19, 1928.

Heifer 1358. Gave birth to small but strong and apparently full-term calf May 5, 1927, 111 days after first exposure and 40 days after the last; afterbirth apparently normal and proved to be negative for *Br. abortus*. Milk on July 11 and 14, 1927, produced lesions of the kind caused by the swine type of *Br. abortus* in guinea pigs; on July 20, 1927, very marked swine-type lesions with orbital abscesses; on October 6, 1927, and January 12 and 28, and February 4, 1928, lesions of the swine type; and on January 7, 1931, more than four years after vaccination, milk still infected with the same type of *Br. abortus*. Calf was killed October 10, 1927; guinea pigs inoculated from portal and mesenteric and pharyngeal glands negative for *Br. abortus*.

Heifer 1372. Gave birth to apparently normal calf February 5, 1927, 22 days after first exposure and before the second was made; afterbirth normal in appearance and negative for *Br. abortus*; milk negative March 22 and 26, April 2 and 6, July 11, 14, and 20, 1927, and May 8, 11, and 16, June 22, 26, and 29, and September 5, 1928. Animal was killed September 5, 1928; supramammary glands negative for *Br. abortus*.

GROUP 3. CONTROL CATTLE

Cow 1174. Gave birth to apparently normal calf June 15, 1927, 152 days after first exposure and 81 days after the last; afterbirth retained but proved to be negative for *Br. abortus*; milk also negative July 11, 14, and 20 and November 22, 1927.

Cow 1338. Aborted June 9, 1927, 146 days after first exposure and 75 days after the last; afterbirth showed characteristic abortion-disease lesions, and was found to be infected with *Br. abortus* as were also the organs and stomach fluid of the fetus; milk negative for *Br. abortus* July 11, 14, and 20 and November 22, 1927, and January 11 and 28, February 25 and 28, and March 3, 1928.

Heifer 1300. Aborted April 4, 1927, 80 days after first exposure and 9 days after the last; afterbirth retained and proved to be infected with *Br. abortus* as were also the organs and stomach fluid of the fetus; and this microorganism was isolated from the lungs, liver, spleen, and stomach fluid of the fetus by cultural methods. Milk proved to be infected April 6 but not infected July 11, 14, and 20.

Heifer 1352. Aborted a fetus, about 7½ months developed, March 22, 1927, 67 days after first exposure and 4 days before the last; afterbirth firmly retained and proved to be infected with *Br. abortus*, as were also the organs and stomach fluid of the fetus; milk infected March 26, April 2, and July 20.

Heifer 1357. Gave birth to full-term calf July 12, 1927, 179 days after first exposure and 108 days after the last; afterbirth passed normally and negative for *Br. abortus*; milk also negative July 14 and 20.

Heifer 1371. Gave birth to apparently normal calf March 26, 1927, 71 days after first exposure and on the day of the last exposure but before it was made;

afterbirth passed normally but showed lesions resembling those of abortion disease and proved to be infected with *Br. abortus*; milk infected April 2 and 6 and July 14, but not infected July 11 and 20 and November 23.

The results of exposing vaccinated and unvaccinated pregnant cows and heifers to *Br. abortus* are shown in condensed form in Table 8.

TABLE 8.—Comparative resistance to *Brucella abortus* exposure of pregnant cows and heifers vaccinated after breeding with different strains of *Br. abortus* and those not vaccinated

Group No.	Cattle	Strain of vaccine	Abortions or premature calves		Cows producing full-term calves		Cows producing infected or noninfected milk		Cows entirely resisting infection	Full-term calves which lived
			Caused by <i>Br. abortus</i>	Not caused by <i>Br. abortus</i>	After-birth infected	After-birth not infected	Infected	Noninfected		
	Number		Number	Number	Number	Number	Number	Number	Per cent	Per cent
1.....	4	Avirulent bovine.....	1	0	2	1	1	3	25	75
2.....	5	Virulent swine.....	0	0	1	4	1	4	60	100
3.....	6	None (control).....	3	0	1	2	3	3	33.3	50

* Heifer 1372, which calved 22 days after a single exposure, is not included but the animal that replaced her is.

† Udder infection caused by vaccination and not by exposure.

EFFECTS OF VACCINATION AND EXPOSURE ON AGGLUTININ CONTENT OF BLOOD

Tables 9, 10, and 11 give the results of agglutination tests made at intervals from the time of vaccination until after the termination of pregnancies. In general the tests were made in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, and 1:800. In a few instances the dilution was carried to 1:1,600 and 1:3,200. In some cases, when it seemed certain that the 1:25 dilution would not furnish additional data, it was omitted as were also the higher dilutions after the data obtained were deemed adequate.

It will be seen in Tables 9 and 10 that agglutinins appeared in the blood of all the vaccinated animals in about a week and had become markedly reduced about 47 days later except in the case of heifer 1358, whose udder became infected with the vaccine.

Attention is called to the close agreement between the blood titers and bacterial findings in Table 10. The titers of the blood of each animal in the group, except the animal whose udder became infected with the vaccine, gradually fell to a low point and did not again appreciably increase.

Table 11 shows that the long-continued exposure had little power to bring out reactions, even in low titer in the two controls that did not become infected, and that the time of appearance of agglutinins after exposure may vary somewhat.

TABLE 9.—*Results of agglutination tests of blood sera of cattle in group 1 (vaccinated with a virulent bovine strain of Brucella abortus during pregnancy) when sera were in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600, and 1:3,200, respectively*^a

Date of vaccination and first and last exposure	Date of agglutination test	Cow 1226	Cow 1361	Heifer 1327	Heifer 1367
1926 Dec. 13, vaccinated....	1926				
	Nov. 22	-----	-----	-----	s-----
	Dec. 13	-----	-----	-----	s-----
	Dec. 16	-----	-----	s-----	s-----
	Dec. 20	-----	o++++ps-	++++s-	o++++ps-
	Dec. 24	o++++s-	o++++s-	+++++	o++++ps-
Five exposures from Jan. 14 to Mar. 26, 1927, inclusive	1927				
	Jan. 4	o+++++s	o++++ps-	-----	o++++s-
	Jan. 14	o++++s-	o++++ps-	o++++s-	o++++s-
	Jan. 26	o++++s-	o++++s-	o++++s-	o++++s-
	Feb. 5	o+ps-	o+ps-	o+ps-	o+ps-
	Feb. 17	o+s-	o+s-	s+p-	o+p-
	Feb. 28	-----	ops-	o+s-	o+p-
	Mar. 7	o++++p-	o+s-	o+s-	o+ps-
	Mar. 14	o++++p-	ops-	o+p-	o+s-
	Mar. 21	-----	o+s-	o+p-	ops-
	Mar. 28	o++++p-	o+++++	o+p-	os-----
	Apr. 2	o++++p-	o++++p-	o+p-	s-----
	Apr. 9	o++++p-	o+++++s	o++++s	+s-----
	Apr. 18	o++++p-	o+++++p	o++++p-	-----
	Apr. 20	-----	^b o+++++	-----	+s-----
	Apr. 26	o++++s-	o++++++	o+++++s	^c +ps-
	May 10	++p-	o+++++	+++++	-----
	May 18	+++p-	o+++++	^d o+++++p	-----
	June 9	o++p-	-----	-----	-----
	June 29	^e o++++	-----	-----	-----
	July 11	+ps-	o++++s-	-----	-----
	Oct. 12	+s-	o++++s-	-----	-----
	1928				
	Jan. 11	o+s---	o++++s-	-----	-----

^a Key: +=complete agglutination; p=partial agglutination; s=slight agglutination; --=no agglutination; o=no test was made in this dilution.

^b Apr. 20, aborted.

^c Apr. 25, calved.

^d May 24, calved.

^e June 27, calved.

TABLE 10.—Results of agglutination tests of blood sera of cattle in group 2 (vaccinated with virulent swine strain of *Brucella abortus* during pregnancy) when sera were in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600, and 1:3,200, respectively.*

Date of vaccination and first and last exposure	Date of agglutination test	Cow 1364	Heifer 1301	Heifer 1349	Heifer 1351	Heifer 1338	Heifer 1372
Dec. 8, 1926, vaccinated	1926 Nov. 22	---	---	---	---	---	---
	Dec. 9	---	---	---	---	---	---
	Dec. 11	---	---	---	s---	---	---
	Dec. 13	---	---	---	+++p	---	---
	Dec. 16	---	+++ps	+++p	+++p	+++s	+++s
Dec. 13, 1926, vaccinated	Dec. 20	+++s	+++p	+++p	+++p	+++s	+++ps
	Dec. 24	+++s	+++p	+++p	+++p	+++s	+++ps
	1927 Jan. 4	+++p	+++p	+++p	+++p	+++s	+++p
	Jan. 14	+++p	+++p	+++p	+++p	+++s	+++p
	Jan. 22	+++p	+++p	+++p	+++p	+++s	+++p
Five exposures from Jan. 14 to Mar. 26, 1927, inclusive	Jan. 26	+++s	+++s	+++s	+++s	+++s	+++s
	Feb. 3	+++s	+++s	+++s	+++s	+++s	+++s
	Feb. 17	+++s	+++s	+++s	+++s	+++s	+++s
	Feb. 26	+++s	+++s	+++s	+++s	+++s	+++s
	Mar. 7	+++s	+++s	+++s	+++s	+++s	+++s
	Mar. 8	+++s	+++s	+++s	+++s	+++s	+++s
	Mar. 14	+++s	+++s	+++s	+++s	+++s	+++s
	Mar. 21	+++s	+++s	+++s	+++s	+++s	+++s
	Mar. 26	+++s	+++s	+++s	+++s	+++s	+++s
	Apr. 2	+++s	+++s	+++s	+++s	+++s	+++s
	Apr. 9	+++s	+++s	+++s	+++s	+++s	+++s
	Apr. 18	+++s	+++s	+++s	+++s	+++s	+++s
	Apr. 26	+++s	+++s	+++s	+++s	+++s	+++s
	May 5	+++s	+++s	+++s	+++s	+++s	+++s
	May 10	+++s	+++s	+++s	+++s	+++s	+++s
	May 18	+++s	+++s	+++s	+++s	+++s	+++s
	June 2	+++s	+++s	+++s	+++s	+++s	+++s
	June 9	+++s	+++s	+++s	+++s	+++s	+++s
	June 10	+++s	+++s	+++s	+++s	+++s	+++s
	June 23	+++s	+++s	+++s	+++s	+++s	+++s
	June 30	+++s	+++s	+++s	+++s	+++s	+++s
	July 11	+++s	+++s	+++s	+++s	+++s	+++s
	July 12	+++s	+++s	+++s	+++s	+++s	+++s
	Oct. 12	+++s	+++s	+++s	+++s	+++s	+++s
	1928 Jan. 11	+++s	+++s	+++s	+++s	+++s	+++s

* Key: ++=complete agglutination; p=partial agglutination; s=slight agglutination; ---=no agglutination; o=no test made in this dilution.
 † Feb. 5, calved.
 ‡ May 5, calved.
 § May 18, calved.
 ¶ June 5, calved.
 †† June 10, calved.
 ‡‡ June 24, calved.
 §§ June 29, calved.

TABLE 11.—Results of agglutination tests of blood sera of cattle in group 3 (controls) when sera were in dilutions of 1:25, 1:50, 1:100, 1:200, 1:400, 1:800, 1:1,600, and 1:3,200, respectively.^a

Date of first and last exposure	Date of agglutination test	Cow 1174	Cow 1338	Heifer 1300	Heifer 1352	Heifer 1357	Heifer 1371
Five exposures from Jan. 14 to Mar. 26, 1927, inclusive.	1926						
	Nov. 22	---	---	---	---	---	s
	1927						
	Jan. 4	---	---	---	---	---	p
	Jan. 14	---	---	---	---	---	os
	Jan. 20	---	---	---	---	---	os
	Jan. 26	---	---	---	---	---	os
	Feb. 3	---	---	---	---	---	---
	Feb. 10	---	---	---	---	---	---
	Feb. 17	---	---	---	---	---	---
	Feb. 24	---	---	---	---	---	---
	Feb. 26	---	---	---	---	---	---
	Mar. 7	---	---	---	---	---	---
	Mar. 14	---	---	---	---	---	---
	Mar. 21	---	---	---	---	---	---
	Mar. 26	---	---	---	---	---	---
	Mar. 28	---	---	---	---	---	---
	Mar. 30	---	---	---	---	---	---
	Apr. 2	---	---	---	---	---	---
	Apr. 9	---	---	---	---	---	---
	Apr. 18	---	---	---	---	---	---
	Apr. 26	---	---	---	---	---	---
	May 10	---	---	---	---	---	---
	May 18	---	---	---	---	---	---
	June 1	---	---	---	---	---	---
	June 9	---	---	---	---	---	---
	June 15	---	---	---	---	---	---
	June 27	---	---	---	---	---	---
	July 11	---	---	---	---	---	---
	July 12	---	---	---	---	---	---
	Sept. 5	---	---	---	---	---	---
	Oct. 5	---	---	---	---	---	---
	Oct. 12	---	---	---	---	---	---
	Nov. 22	---	---	---	---	---	---
	Dec. 16	---	---	---	---	---	---
	1928						
	Jan. 11	---	---	---	---	---	---
	Mar. 3	---	---	---	---	---	---

^a Key: + = complete agglutination; p = partial agglutination; s = slight agglutination; -- = no agglutination; o = no test made in this dilution.

^b Mar. 22, aborted.

^c Apr. 4, aborted.

^d June 9, aborted.

^e June 15, calved.

^f July 12, calved.

SUMMARY AND CONCLUSIONS

Experiments were made to determine the efficacy of three strains of *Brucella abortus* as immunizing agents against infectious abortion. The strains tested were an avirulent bovine strain, a virulent swine and a virulent bovine strain. The results of these experiments indicate that the strain of bovine *Br. abortus* avirulent for guinea pigs or the virulent swine strain, when administered to cows and heifers three to five and one-half months before service, afforded only slight protection against repeated exposure to *Br. abortus* as compared with that induced by the virulent bovine strain. When given after conception the avirulent bovine strain seemed to increase resistance to the progress of the disease and the swine strain appeared to give a definite immunity. However, the latter strain is likely to infect the udder for long periods and hence its use for vaccinating cattle, because the swine strain of *Br. abortus* is generally believed to be more dangerous to human health than the bovine, is out of the question.

These findings throw light on the relation of swine strains of *Br. abortus* bovine and show the danger of infecting the udder with a swine strain of *Br. abortus* if it should, by accident, find its way into vaccine.

Neither the avirulent bovine nor the virulent swine strain of vaccine, administered from three to five and one-half months before breeding, produced much more than a slightly greater resistance to the long-continued severe exposure given than that possessed by the controls; nor did either of the vaccines prevent the udders from becoming infected. In one instance the swine strain of vaccine caused udder infection.

A vaccine prepared from virulent bovine *Br. abortus* afforded a marked resistance to abortion and uterine infection under the same conditions as those of the two previously mentioned strains but either did not prevent udder infection or was the direct cause of it in half the animals that completed the experiment. Udder infection, however, was greater among the controls. The accidental use of this vaccine on a pregnant heifer caused her to abort.

Brucella abortus of the swine type, when used as vaccine before conception, may invade the udder and associated lymph glands and persist there for at least one and one-third years, and if administered to pregnant cattle may also invade the udder and persist there, without appreciable change in character, for at least four years.

DISTRIBUTION OF THE COTTON ROOT-ROT FUNGUS IN SOIL AND IN PLANT TISSUES IN RELATION TO CONTROL BY DISINFECTANTS¹

By C. J. KING, *Agronomist*, and CLAUDE HOPE, *Junior Horticulturist*, Division of Cotton, Rubber, and Other Tropical Plants, Bureau of Plant Industry, United States Department of Agriculture²

INTRODUCTION

The root-rot disease caused by the fungus *Phymatotrichum omnivorum* (Shear) Dug. is responsible for heavy losses in many of the economic plants grown in the Southwestern States. This fungus has a wide range of host plants but is less destructive to some species than to others. Some species possess powers of resistance apparently because of some peculiar histological structure of the roots, and others contain certain chemical substances which seem to repel the invasion of the fungus. Some of the trees and shrubs, because of their habit of developing extensive root systems, are able to resist the disease after attaining some degree of maturity. Even those that are highly susceptible often live for several months after becoming infected, and certain individuals may live for years because of some unusual distribution of their roots, which allows part of them to escape infection. On account of these differences in resistive powers, it is not always possible to determine from the appearance of the tops whether the plants are infected.

It is often desirable for the plant grower or the investigator to determine the actual limits of areas that are infested with the root-rot fungus. Where field crops such as alfalfa or cotton are grown continuously for several years this may be accomplished fairly accurately by mapping periodically the areas of dead plants. In plantings of trees and shrubs or in fallow land, however, different methods are required.

Incidental to an attempt to eradicate the root-rot fungus by the use of formaldehyde in an isolated area of approximately 5 acres, near Indio, Calif., in 1930, some information was secured which would appear to be of value in relation to any control methods or investigations that are undertaken. The practicability of mapping infested areas was indicated even where no infected plants were present to serve as indicators. It was also brought out that hyphae that had gained entry into the interior tissues of large woody roots were afforded protection against disinfectants for a long period.

The discovery in 1928 of a sclerotium stage in the life history of the root-rot fungus led to an intensified study on the part of several investigators to determine the character of the sclerotial structures and their relation to the dissemination of the disease and its carry-over from one season to another.

The production of sclerotia in pure cultures at Sacaton, Ariz., in 1928, was first reported by King and Loomis (3)³ in 1929. Neal (6)

¹ Received for publication Mar. 5, 1932; issued December, 1932.

² The writers are indebted to H. F. Loomis, associate agronomist, and Orlan Parker, assistant scientific aide, of this division, and to F. A. Thackery, senior agriculturist, and Dewey C. Moore, scientific aid, of the Division of Horticultural Crops and Diseases, for valuable assistance in conducting these investigations.

³ Reference is made by number (italic) to Literature Cited, p. 740.

and later Dana (1) and Taubenhause and Ezekiel (8) reported the finding of sclerotia under natural conditions in the soil in Texas. Further contributions by Taubenhause and Ezekiel (9), Dana (2), and King, Loomis, and Hope (4) have furnished additional information on the properties of sclerotia. Some investigations conducted in Arizona in 1930 and 1931 on the distribution of sclerotia in soil where various types of host plants were grown are reported as a part of this paper.

METHOD OF MAPPING INFESTED AREAS

Soon after the identification of the root-rot fungus in an isolated area near Indio, Calif., in 1928, some preliminary measures were taken to prevent further spread.

Before an adequate survey was made to delimit the infested area, trenches were dug a few feet outside the supposed limits of the infested spots, with the idea that they might serve as barriers against further spread of the fungus. Only such areas were included within the trenches as showed the disease by aboveground symptoms on the susceptible plants.

It soon became apparent that many of the trenches were within the infested area. When moisture conditions in the trenches became favorable, spore mats appeared in many of them, even at some distance from growing plants. (Fig. 1.) On part of the area a second line of trenches was later dug farther out, but in these also spore mats appeared in some places. Although the open trenches were not infallible guides to the presence of the disease where no plants were present, their value in this respect was well demonstrated.

When it was decided to treat the entire infested area with a disinfectant, a survey was made by excavating and examining the roots of plants in areas where the presence of the disease was suspected. The inspectors were aided in this work by the fact that the entire area of about 5 acres was more or less permeated by the roots of susceptible trees. "In parts of the area, the trees themselves were still standing, and in other places they had been removed some months or even years previously. It was possible to find living or dead roots in almost every excavation.

By making a large number of excavations and charting each spot in which infected roots were found, it was possible to obtain a fairly good idea of the limits of the disease; it was discovered that the infestation involved an almost continuous area instead of being confined to disconnected spots as indicated by the dead trees and crop plants.

Two parallel rows of large pistache trees (*Pistacia atlantica* Desf.) showed no evidence of disease aboveground, but excavations proved that two trees at each end of the avenue had infected roots, parts of which extended into an adjacent date orchard. Similar evidence was found along a hedge of large athel trees (*Tamarix aphylla* (L.) Karst.). No direct evidence was found that the athel roots were attacked, but the presence of the fungus on the surface of some of the roots and the occurrence of the disease in test crops in adjacent land beyond the athel hedge indicated that the roots may have served as a host. The roots of other species of *Tamarix* in a mixed planting which formed a hedge about 100 feet long at the northwest corner of the garden, were found to be seriously infected, although no trees had died. Large portions of the roots of these trees throughout the length of the



FIGURE 1.—Conidial mats of the root-rot fungus forming in a trench near the margin of an infested orchard near Indio, Calif.

hedge were rotted away and showed many fragmentary strands of *Phymatotrichum*.

Under the conditions at Indio the fungus apparently may exist in an active state for a long time on the roots that are left in the soil after the aerial parts are removed. The experience in this case suggests the great importance of learning the history of an infested area before planning experiments or attempting control measures, and indicates the feasibility of detecting the presence of the disease in the absence of indicator plants by a proper survey and examination of the roots within the suspected area.

CONDITIONS INFLUENCING THE EFFECTIVENESS OF DISINFECTANTS

Experiments conducted in an attempt to eradicate spots of root rot by applying disinfectants to the soil brought out the fact that the mycelium that had penetrated into the inner tissues of roots was protected against disinfectants and that this protection offered one of the most serious obstacles to the success of control measures. The existence of protected hyphae was first recognized in making cultures of the root-rot fungus from interior tissues of large roots at Indio, Calif.

Where the soil is permeable to solutions, sclerotia and exposed strands are readily destroyed by formaldehyde (4). Laboratory tests showed that the germinating power of sclerotia of average size (about 2 mm) was destroyed when they were immersed in a 1 per cent solution of commercial formaldehyde (formalin) for about 45 minutes. The sclerotia were likewise killed after 3 hours when placed 6 inches deep in cans of soil that had been recently treated with a 1 per cent solution of formalin.

On January 29, 1931, about 70 sclerotia, from 2 to 4 mm in diameter, were removed from four or five borings in part of the area at Indio that had been treated about 10 months previously with a 1¼ per cent solution of formalin. The majority of these sclerotia were soft and shrunken and in a state of decomposition. About 20 of the largest and most turgid individuals were tested for germination, but all of them failed to grow. On June 2, 1931, 118 sclerotia from the treated area were examined, and all proved to be dead; most of them were badly decayed.

The mycelium that had penetrated the tissues of plant roots, however, was not so readily destroyed. A period of 6 hours was required to kill the hyphae in sections of fig (*Ficus carica* L.) and chinaberry (*Melia azedarach* L.) roots, one-half to three-fourths inch in diameter, immersed in a 1¼ per cent solution of formalin. A period of 12 hours was required to kill the mycelium in ½-inch cotton roots placed in a soil that had been treated with a 1 per cent solution of formalin.

At Indio the attempt to eradicate the root-rot fungus was carried out under the direction of F. A. Thackery in cooperation with the California State Department of Agriculture (5). The method consisted in introducing a 1¼ per cent formalin solution under pressure by means of injector nozzles on the ends of ¾-inch gas pipes, which were inserted gradually into the soil to a depth of 6 feet. (Fig. 2.) The injections were made 1 foot apart, and 6 gallons of solution was introduced at each injection.

The soil in the infested area consists of a very fine sand that extends to great depths; its permeable character makes it especially favorable for the uniform distribution of the solution.

In order to obtain some idea of the effectiveness of the treatment against mycelium that had penetrated the inner tissues of roots in the soil, large numbers of root sections of fig, pistache, and chinaberry, from three-eighths to 1 inch in diameter, were sterilized in an autoclave and infected with the root-rot fungus. After the fungus had developed and maintained a profuse growth for about 5 days, each section was tagged, some were wrapped in filter paper, and all of them were inserted in the soil at depths of from 1 to 7 feet. (Fig. 3.) The formalin solution was injected over them in the regular manner, after which specimens were removed at intervals of 1 to 30 days and tested to determine whether the fungus remained alive. A revived growth of the fungus resulted from most of the root sections removed during the first 12 days after treatment, but only 25 to 30 per cent of those that were left in the soil from 12 to 30 days showed further root-rot growth.

In another test, fig and chinaberry roots $1\frac{1}{2}$ to 2 inches in diameter were cut into sections about 3 inches long. A hole about 1 inch deep was made in one end of each with a $\frac{3}{8}$ -inch drill bit. After the sections had been sterilized, root-rot infected root tissues were inserted in the holes, which were then stoppered tightly with corks, and the stoppered ends sealed with paraffin. (Fig. 4.) They were then inserted in the soil in the manner described above and the formaldehyde treatment applied.



FIGURE 2.—Introducing formalin solution into an infested area in an effort to eradicate the root-rot fungus

Specimens were removed from the soil for examination at intervals varying from 10 to 30 days. The fungus was still alive in all the specimens; in most cases it was found that the mycelium had grown through the conducting vessels of the roots and had escaped at the unsealed end of each section, where it developed a dense growth that in some cases almost covered the entire root section. Contaminations were found on some of the sections, but the root-rot mycelium was the predominating growth.

This behavior is in accord with the observations of Peltier, King, and Samson (7), who reported the presence of hyphae in the outer xylem vessels of alfalfa roots 50 cm beyond the diseased portions. Under natural conditions it is quite possible that hyphae find their way through deep lesions or wounds into the conducting vessels of many woody roots and extend far into the healthy tissues. Under such conditions they would probably be protected for long periods against any disinfectants that might be applied to the soil.

On January 29, 1931, a large number of roots and root fragments were exhumed in the area that had received the formaldehyde treatment about 10 months previously. For the most part they were in a soggy condition unfavorable to the growth of the fungus. Fragments of strands were still visible on the surface of some, but these appeared to be dead and in a state of decomposition.



FIGURE 3.—Cultures of the root-rot fungus on roots of chinaberry and of fig. These were placed in the soil at various depths to test the effects of the formalin solution



FIGURE 4.—Roots of fig and chinaberry with root-rot inoculum stoppered inside the tissues. These were placed in the soil at various depths to test the effects of the formalin solution

VIABILITY AND PROPAGATIVE POWERS OF SCLEROTIA

In a previous publication, King, Loomis, and Hope (4) reported successful inoculation of cotton plants with sclerotia 11 months old and described a budding process by which the viability of sclerotial masses was believed to be prolonged.

On May 6, 1931, a test was made to determine whether any of the sclerotia were viable in a culture jar of sand and cotton roots that had been kept in the laboratory since the sclerotia were first noted on October 29, 1928, over two and one half years before. Water had

been added to this jar about eight months after the culture was prepared, but after that it had received no attention. A little moisture was present in the sand at the time the sclerotia were tested. A few of the sclerotia showed signs of decomposition, but the majority were still well preserved. Thirty-one of the latter were placed on moist filter paper in Petri dishes and incubated. Within a few days all of them had germinated and produced a vigorously growing mycelium.

In December, 1930, a test was conducted to determine whether sections and fragments of sclerotia possessed the powers of germination and growth shown by entire individuals. Taubenhaus and Ezekiel (8) reported that the same sclerotia would produce growth as many as five times after the previous growth had been removed, and it seemed possible that hyphal growth might issue from almost any part of the structures. Accordingly, 40 large, well-developed simple sclerotia that had recently been taken from infested soil were selected for the test. After they had been washed in mercuric chloride solution and sterile water they were divided into three lots. From each of the 20 sclerotia in lot 1 the thin outside coat was carefully cut away, so that as little as possible of the inner tissue was removed in the process. From the 5 individuals in lot 2, thin cross sections were made with a scalpel. Ten of these sections and 42 of the fragments removed from the first group were then selected at random for testing.

Lot 3, consisting of the remaining 15 untreated sclerotia, was used as a check. All three lots were then placed on sterilized moist filter paper in Petri dishes and incubated. Within a few days, typical root-rot strands had grown from 19 of the 20 sclerotia from which the outside tissue had been removed, the 52 fragments and sections had germinated, and the 15 sclerotia used as a check had produced vigorous growth. (Fig. 5.) The growth from the fragments and "peeled" sclerotia was hardly as vigorous as that from the unmutated ones, but undoubtedly there would have been no difficulty in establishing cultures or in obtaining infection from this material. Later this experiment was repeated, and the results were practically the same.

DISTRIBUTION OF SCLEROTIA IN INFESTED AREAS

In 1928 repeated search was made for sclerotia in the soil of infested areas at Sacaton, Ariz. After several months some were found, but only in parts of some areas and not at all in others. In most areas the color of the sclerotia was so similar to many of the soil particles that it was not practicable to distinguish between them in examining samples of dry soil by ordinary methods of dissection. A method was devised which made it practicable to separate the sclerotia from the soil particles so that they could be counted and estimates made of the number in an infected area.

The procedure was as follows: Soil samples of equal volume were removed from definite zones at various depths by means of a trowel or post-hole auger. Each sample was placed in the top of a set of two sieves, each 12 inches square. The bottom of the upper sieve was made of hardware cloth of $\frac{1}{4}$ -inch mesh, and that of the lower sieve of 20-mesh screen wire. (Fig. 6, A.) The finer soil particles were forced through the screens by water under pressure from a hose nozzle, leaving the larger particles, together with root fragments, on the top

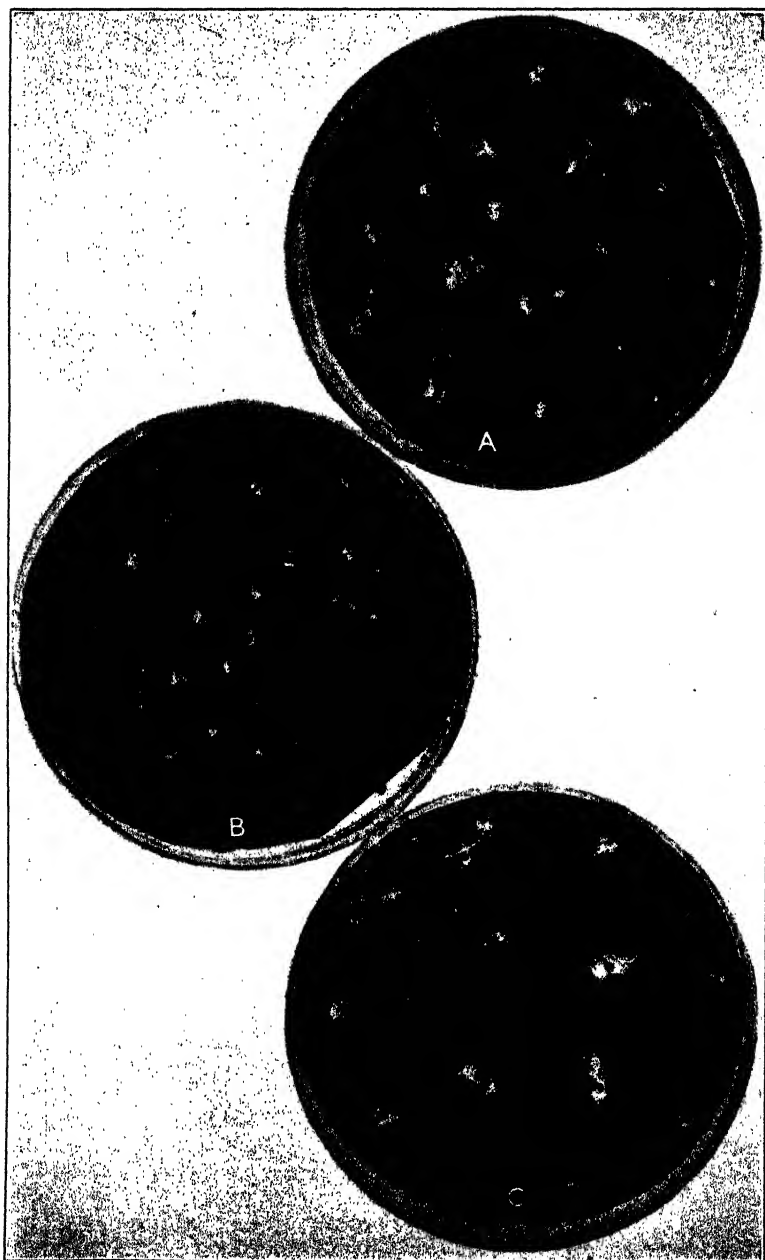


FIGURE 5.—Germination of root-rot sclerotia: A, 20 sclerotia developing vigorous mycelial growth after removal of outside covering; B, sections of inside tissues and fragments of the covering or "peel" producing growth of root-rot mycelium; C, 15 whole sclerotia germinating to produce growth of root-rot mycelium

screen, and the sclerotia, with coarse sand and other débris, on the bottom screen. The sclerotia could then be picked out easily with forceps. (Fig. 6, B.)

When this method was first adopted, the soil was removed in layers 4 inches thick and 12 inches square. In an infested cotton field examined in August, 1930, the 4-inch blocks were obtained in duplicate to a depth of 24 inches, and the two from the same depth were combined to form two-thirds of a cubic foot of soil. This was placed on the screens, and the sclerotia were separated by the washing process. The number of sclerotia contained in each 4-inch layer taken at depths of from 8 to 24 inches, was as follows:

Depth in inches	Number of sclerotia
4-8	0
8-12	11
12-16	226
16-20	632
20-24	152



FIGURE 6.—A, Equipment used in separating root-rot sclerotia from the soil to determine their size and number in infested areas; B, part of residue of coarse sand and sclerotia left on bottom screen after a 13-pound sample of infested soil had been washed through the screens. The sclerotia are usually much darker than the soil particles and can be readily picked out from the residue with forceps. B about one-half natural size

So many sclerotia were found in the layer between the depths of 20 and 24 inches that probably other sclerotia would have been found below the latter depth if the excavations had been carried farther.

In cultivated land, sclerotia were seldom found at a depth of less than 4 to 6 inches; therefore in most of the sampling operations, the topsoil to that depth was discarded.

In January, 1931, several borings were made for soil samples in an infested area in an alfalfa field near Coolidge, Ariz. The soil in this area is of the Mohave sandy loam type, containing much gravel and a high proportion of calcareous material locally known as caliche. The spot selected was almost semicircular in shape and about 20½ feet in longest diameter. It was bordered by a fence separating two fields of alfalfa. A few of the alfalfa plants across the fence from the spot showed signs of infection, but the limits of infestation were

not well defined. Six borings (Nos. 1 to 6) were made with a 7-inch posthole auger on the periphery of the circular spot along the margin of dead plants. (Fig. 7.) Hole 9 was located near the center, holes 7 and 8 midway between the center and the periphery, and hole 10 among live plants a few inches beyond the fence in the other field.

On a later date three holes, designated as 1-A, 2-A, and 3-A, were made outside the margin of dead plants opposite holes 1, 2, and 3, respectively, so that their centers were 10 inches from the centers of the holes previously made. Three others, designated as 1-B, 2-B, and 3-B, were located still farther out among the living plants, so

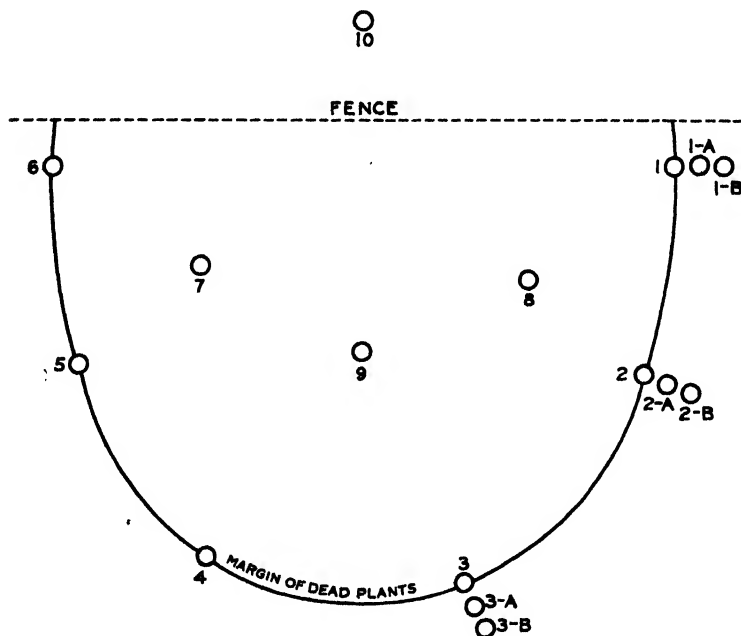


FIGURE 7.—Diagram of a spot of root-rot infection in alfalfa, near Coolidge, Ariz., showing the location of the holes from which soil samples containing sclerotia were removed with a 7-inch posthole auger. Six holes (Nos. 1-6) were located along the margin of dead plants; hole 9, near the center; holes 7 and 8, midway between the center and the periphery; hole 10 among live plants; holes 1-A and 1-B; 2-A and 2-B, and 3-A and 3-B, among live plants, with centers 10 and 20 inches from the centers of holes 1, 2, and 3, respectively. (Table 1.) Scale, 1 inch=6 feet

that their centers were 20 inches beyond the centers of holes 1, 2, and 3 on the margin. After the top 6 inches had been discarded, the soil from each 6-inch depth, representing about 13 pounds of soil, was placed in separate containers and conveyed to the laboratory for separation of the sclerotia by the washing process. Some of the holes were dug only 30 inches deep, but others were sampled to a depth of 42 inches, and holes 2, 3, and 9 were 54 inches deep. The number of sclerotia found at different 6-inch depths in these first 10 holes that were dug is given in Table 1. Since only 12 were found in the holes that were dug later, outside the area of dead plants, the results are not included in the table. These occurred at a depth of 6 to 18 inches in hole 3-A.

TABLE 1.—*Distribution of root-rot sclerotia in an infected spot of alfalfa near Coolidge, Ariz.*

Depth (inches)	Number of sclerotia in hole No. —										Total
	1	2	3	4	5	6	7	8	9	10	
0-12.....	53	58	5	287	0	1	2	101	1	2	510
12-18.....	51	305	^b 212	163	12	1	0	171	129	13	1,057
18-24.....	6	14	15	17	6	0	0	211	690	4	969
24-30.....	18	1	7	2	1	0	1	157	282	6	475
30-36.....	5	1	0	—	—	—	—	—	161	—	167
36-42.....	0	0	0	—	—	—	—	—	140	—	140
42-48.....	—	0	0	—	—	—	—	—	0	—	0
48-54.....	—	0	0	—	—	—	—	—	4	—	4
Total.....	133	379	239	469	19	2	3	640	1,407	25	3,316

^a See fig. 7.^b 5 of these sclerotia were dead.

While some sclerotia were found in each of the 10 holes, they were unevenly distributed throughout the soil. The greatest number was obtained in hole 9, which was in the center of the spot. The eight samples from this hole gave a total of 1,407 sclerotia. These were darker in color than most of those from other holes, indicating that they were older, but they were all viable. Judging by the size of the spot, they must have developed at least a year previously, when the disease first became active at the center. From the large number of sclerotia present in some holes at depths of 6 to 12 inches, it is probable that there were a few in the top 6 inches of soil that was discarded. In all the borings the sclerotia were most abundant at depths of 6 to 24 inches. In hole 9 a considerable number were found at a depth of 30 to 42 inches, and four occurred at a depth of 48 to 54 inches.

During April and May, 1931, soil samples were taken from several parts of an infested area in a Bermuda-grass lawn at the United States Field Station at Sacaton, Ariz. In July, 1930, an 11-year-old Chinese elm (*Ulmus pumila* L.) located in this area died suddenly from root rot and was dug out. The main laterals were cut a few inches from the taproot and were left in the soil. Except for another Chinese elm, located about 15 feet distant, there were no other susceptible plants near by. The soil in this area is a Gila fine sand that is fairly uniform throughout the depth sampled. In sampling, the top 3 inches, consisting largely of Bermuda-grass sod, was discarded. The soil from a depth of 3 to 6 inches comprised the first sample; below 6 inches, the samples were taken in 6-inch layers.

In most of the borings the samples were taken to a depth of 3 to 7 feet with a 7-inch posthole auger. Samples from lower depths were taken with a 6-inch posthole auger. This was done to prevent the sclerotia from being dislodged from the sides of the holes at upper levels and thus becoming mixed with samples from greater depths. As a further precaution, the 6-inch auger was operated on the inside of an iron pipe having a diameter slightly larger than that of the auger. As the depth of the hole increased the pipe was pressed gradually into the soil just behind the cutting edge of the auger, and the samples were drawn upward through the pipe without making contact with the soil on the sides of the hole.

A diagram showing the location of all the holes with reference to the tree is given in Figure 8. The numbers of sclerotia found at different depths in the nine holes made in this area are given in Table 2.

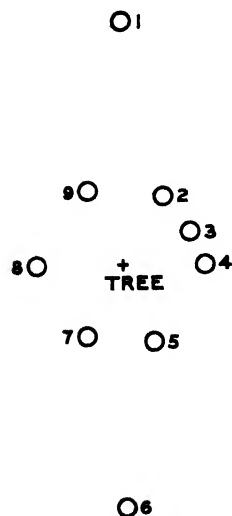


FIGURE 8.—Diagram showing position of holes (Nos. 1-9), from which soil samples containing root-rot sclerotia were obtained with a posthole auger, with reference to the location of a Chinese elm that had died from root rot. (Table 2.) Scale, 1 inch=6 feet

In this, as in the other areas sampled, the sclerotia were unevenly distributed and appeared to concentrate more or less in pockets of soil.

In holes 5 and 8 the sclerotia were most abundant at a depth of 6 to 18 inches; in hole 9, at a depth of 18 to 30 inches, and in hole 1 at a depth of 30 to 42 inches. In hole 2 very few were obtained above a depth of 36 inches, but a large number occurred at greater depths. No sclerotia were found in hole 6, which was located 8 feet south of the tree, but 892 were obtained from hole 1, 8 feet north of the tree.

In holes 5 and 9 a few sclerotia were found in the samples removed from a depth of 84 to 90 inches. Their occurrence at practically all the 6-inch levels above that depth indicates that their presence at this great depth was not the result of accident in sampling. Live root-rot strands on diseased roots were also found at that level, and this gave further weight to the evidence that the sclerotia actually developed 84 to 90 inches below the surface of the soil.

It was found that a sample of soil taken from the lower levels with the 6-inch auger weighed about 13 pounds, which was about the same as a sample of the same thickness taken near the surface with a 7-inch auger.

TABLE 2.—Distribution of root-rot sclerotia in an infested area where a Chinese elm tree had died from the disease at Sacaton, Ariz., in 1930

Depth (inches)	Number of sclerotia in hole No.—									Total
	1	2	3	4	5	6	7	8	9	
3-6.....	0	0	0	-----	0	0	13	0	-----	13
6-12.....	12	0	0	4	838	0	36	882	5	1,777
12-18.....	26	2	^b 3	10	81	0	142	744	61	1,069
18-24.....	75	18	^c 49	64	33	0	168	48	774	1,229
24-30.....	100	5	71	38	18	0	21	6	856	1,121
30-36.....	277	0	53	19	98	0	8	3	108	566
36-42.....	346	77	^d 3	809	28	0	32	6	154	1,455
42-48.....	^d 39	338	1	141	11	0	59	3	69	661
48-54.....	11	12	5	5	11	0	55	2	14	115
54-60.....	0	32	4	9	8	0	7	^d 3	4	67
60-66.....	0	58	0	^d 0	3	0	7	^d 4	3	75
66-72.....	0	18	0	0	4	0	3	0	0	25
72-78.....	0	3	0	0	1	0	3	0	0	10
78-84.....	0	2	0	0	2	-----	11	0	1	16
84-90.....	-----	^d 0	-----	-----	^d 1	-----	^d 0	-----	^b 3	4
90-96.....	-----	0	-----	-----	0	-----	-----	-----	0	0
96-102.....	-----	-----	-----	-----	0	-----	-----	-----	0	0
Total.....	892	565	189	1,099	1,137	0	565	1,701	2,055	8,203

^a See Fig. 8.

^b Two of these sclerotia were dead.

^c Seven of these sclerotia were dead.

^d The sheet-iron pipe and 6-inch auger were used in removing this and lower samples from this hole.

^e Three of these sclerotia were dead.

Of the nine holes that were made in sampling, seven were located roughly in a circle approximately $2\frac{1}{2}$ feet from a center representing the site of the tree trunk. One hole (No. 1) was made 8 feet north and another hole (No. 6) 8 feet south of the same central point.

The total number of sclerotia obtained in all samples from the nine holes was 8,203. Of these, 7,311 were obtained from the seven holes (Nos. 2, 3, 4, 5, 7, 8, and 9) located on the periphery of a circle with a radius of approximately 30 inches. (Fig. 8.) Using the average of

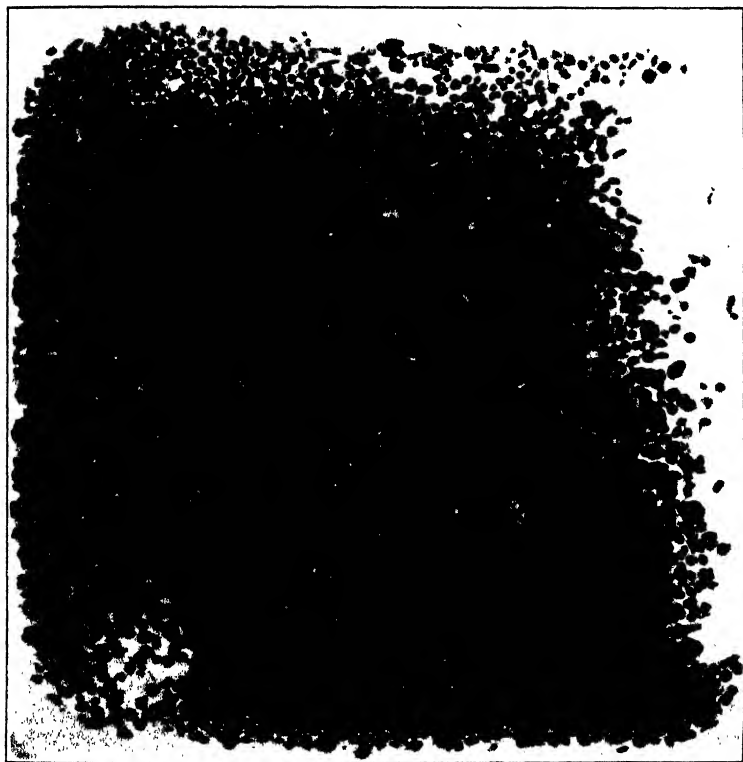


FIGURE 9.—A mass of sclerotia (approximately 5,000) obtained from soil samples removed in nine borings in an area where a Chinese elm had died from root rot. Natural size

these holes as a basis, it is estimated that there were about 100,000 sclerotia within this circle. A mass of about 5,000 sclerotia is shown in Figure 9.

The amount of soil examined was approximately 1,865 pounds. It was estimated that 1 cubic foot weighed 100 pounds, and if it is assumed that the root system of the tree occupied an area of 400 square feet and extended to a depth of 6 feet and that the entire volume harbored a population of sclerotia proportionate to that in the samples, the number of sclerotia involved would be 1,056,616. On this basis, the number of sclerotia in an acre would be 114,956,584.

It has been learned from experience in conducting germination tests with sclerotia that their color grows darker with age and that those which have become soft or flaccid will not germinate. Of the 8,203 sclerotia obtained from the nine borings, 12 were soft and so disintegrated that no test was considered necessary to prove that they were not viable. A large number of the others, including some of the darkest specimens, were tested, and all germinated.

Since the tree was killed about nine months previously and the area had doubtless been infested for some time before that, it is evident that some of the sclerotia must have been at least a year or more old when tested. It would appear, therefore, that they do not lose their viability very rapidly under natural conditions in the soil.

SUMMARY AND CONCLUSIONS

In an attempt to fix the limits of an area infested with the cotton root-rot fungus, *Phymatotrichum omnivorum* (Shear) Dug., near Indio, Calif., methods were developed which served as guides in detecting the presence of the fungus even where no indicator plants were present.

When conditions of moisture were favorable, conidial mats of the fungus formed in great abundance in open trenches that transected the infested area. Numerous trenches dug near the supposed margins of the infested area aided in locating the actual limits of the disease.

In areas where the roots of susceptible plants were abundant, a fairly good idea of the limits of the disease was obtained by making numerous excavations throughout the area and inspecting the root fragments for the presence of the fungus.

It was found that the roots of several large pistache trees (*Pistacia atlantica*) were infected, although the trees showed no symptoms of the disease above ground. Root-rot strands were found on the surface of some of the roots of large athel trees (*Tamarix aphylla*), but no injury to the roots was observed. In other species of *Tamarix* the roots showed extensive damage from the fungus, but the trees remained alive.

It is evident that the root-rot fungus is able to exist in an active state for a long period on buried roots that remain in the soil after trees have died or have been removed.

The root-rot mycelium that had penetrated into the inner tissues of large roots was protected for a long period from disinfectants that readily destroyed exposed strands and sclerotia.

In attempting to eradicate the fungus from the area near Indio, a 1¼ per cent solution of formalin (commercial formaldehyde) was injected into the soil, by pressure through ¾-inch pipes, to a depth of 6 feet, at the rate of 1 gallon per cubic foot. To test the effectiveness of this treatment, pure cultures of the root-rot fungus growing on sterilized sections of fig, chinaberry, and pistache roots three-eighths of an inch to 1 inch in diameter were buried from 1 to 7 feet deep in the soil, and the formalin solution was applied over them in the usual way. A majority of the root specimens that were removed from the soil and tested during the first 12 days after the formaldehyde treatment showed a renewed growth of the fungus, but only 25 to 30 per cent of those that remained in the soil 12 to 30 days showed further root-rot growth.

In other tests root-rot inoculum was placed on the inside of steam-sterilized sections of fig and chinaberry roots $1\frac{1}{2}$ to 2 inches in diameter and 3 inches long. These were sealed at one end and buried in the soil at depths of 1 to 7 feet immediately before the formaldehyde treatment was applied. When specimens were removed and examined 30 days after treatment, the inoculum in all the sections was alive, and in some specimens the mycelium had grown through the conducting vessels and partly covered the outside tissue.

Examination of a large number of roots and root fragments removed from the formaldehyde-treated area, about 10 months after the treatment, indicated that all the mycelium on the outside tissues was dead.

In soil samples taken from the formaldehyde-treated area, about 10 months after the treatment, 70 root-rot sclerotia were obtained; in other samples, taken 12 months after the treatment, 118 were obtained. All these were found to be dead.

Root-rot sclerotia from a culture kept in the laboratory for two and one-half years germinated and produced a vigorously growing mycelium.

Laboratory tests showed that all parts of a root-rot sclerotium are capable of producing mycelium. Specimens from which the outer coats had been removed, fragments of the outer coat, and thin cross sections of sclerotia, all produced mycelial growth.

In order to facilitate the separation of sclerotia from the soil of infested areas a sieve made of $\frac{1}{4}$ -inch mesh hardware cloth was placed over another sieve of 20-mesh screen wire. The soil was washed through them, leaving the coarsest soil and root material in the top sieve, and the sclerotia and some of the coarse material in the bottom sieve.

In 1931 a small infested area in an alfalfa field near Coolidge, Ariz., and another on a Bermuda-grass lawn at Sacaton, Ariz., where a Chinese elm had been killed the previous year, were sampled with a posthole auger, and the sclerotia were separated from the soil by the washing process and counted.

In the Coolidge area the sclerotia were most abundant at a depth of 6 to 24 inches, but four were found 48 to 54 inches below the surface. In the Sacaton area the sclerotia were more concentrated at various depths between 6 and 42 inches, but a few were found at a depth of 84 to 90 inches.

It is apparent that the distribution of sclerotia is very uneven, both vertically and horizontally, in infested soils.

In soil samples taken from nine borings in an area where a Chinese elm had died, 8,203 sclerotia were obtained. It was estimated that the total number of sclerotia in the soil comprising the root zone of the tree was more than a million. That most of the sclerotia were alive in an area that was known to have been infested for several months indicates that sclerotia under natural conditions do not soon lose their viability.

LITERATURE CITED

- (1) DANA, B. F.
1929. RECENT DEVELOPMENTS IN ROOT-ROT INVESTIGATIONS. *Farm and Ranch* 48 (46): 22-23, illus.
- (2) ———
1931. SOIL CULTURES FOR THE LABORATORY PRODUCTION OF SCLEROTIA IN PHYMATOTRICHUM OMNIVORUM. *Phytopathology* 21: 551-556, illus.
- (3) KING, C. J., and LOOMIS, H. F.
1929. FURTHER STUDIES OF COTTON ROOT-ROT IN ARIZONA, WITH A DESCRIPTION OF A SCLEROTIUM STAGE OF THE FUNGUS. *Jour. Agr. Research* 39: 641-676, illus.
- (4) ———, LOOMIS, H. F., and HOPE, C.
1931. STUDIES OF SCLEROTIA AND THE MYCELIAL STRANDS OF THE COTTON ROOT-ROT FUNGUS. *Jour. Agr. Research* 42: 827-840, illus.
- (5) MILBRATH, D. G.
1929. PLANT PATHOLOGY. *Calif. Dept. Agr. Mo. Bul.* 18: 774-777.
- (6) NEAL, D. C.
1929. THE OCCURRENCE OF VIABLE COTTON ROOT-ROT SCLEROTIA IN NATURE. *Science* (n. s.) 70: 409-410.
- (7) PELTIER, G. L., KING, C. J., and SAMSON, R. W.
1926. OZONIUM ROOT ROT. *U. S. Dept. Agr. Bul.* 1417, 28 p., illus.
- (8) TAUBENHAUS, J. J., and EZEKIEL, W. N.
1930. STUDIES ON THE OVERWINTERING OF PHYMATOTRICHUM ROOT ROT. *Phytopathology* 20: 761-785, illus.
- (9) ——— and EZEKIEL, W. N.
1930. RECENT STUDIES ON PHYMATOTRICHUM ROOT-ROT. *Amer. Jour. Bot.* 17: 554-571, illus.

DETERMINATION OF THE ERRORS OF ESTIMATE OF A FOREST SURVEY, WITH SPECIAL REFERENCE TO THE BOTTOM-LAND HARDWOOD FOREST REGION¹

By FRANCIS X. SCHUMACHER, *Silviculturist, Division of Silvics*, and HENRY BULL, *Assistant Silviculturist, Southern Forest Experiment Station, Branch of Research, Forest Service, United States Department of Agriculture*

INTRODUCTION

The Forest Service, under the authorization of the McSweeney-McNary Forest Research Act, is at present undertaking an exhaustive survey of the country's forest lands, by comparatively small natural or political subdivisions, such as groups of counties, with the purpose of obtaining therefrom a complete and accurate picture of the forest situation and the forest needs of the Nation. Chief among the required results of this survey are the following: Area in each type of forest cover, subdivided according to present productive condition; volume of timber by species; rate of growth on existing forests and reforesting areas; rate of forest depletion by cutting, fire, insects, and other agencies; and present national timber requirements and probable future trends.

The diversity of timber types, forest conditions, and timber growth to be encountered in such a survey, together with the scanty knowledge we now have of these in quantitative terms, make it imperative, in order to carry out the project efficiently with the funds available, that methods of gathering and compiling the enormous amount of data be carefully worked out in advance for each major forest region. This is especially important in view of the fact that the bulk of the data must be collected at first hand in the field.

Of first importance is a method of estimating the extent of data that must actually be gathered in any one large unit of area, and the nature of the survey, in order to obtain a reliable basis for estimating accurately the condition of the entire unit. The object of this paper is to present, by means of the analysis of a comparatively small set of field data gathered for this purpose from the bottom-land hardwood forest region, a practical means of determining these particulars for any given region and of making certain that the probable error of the results, for groups as small as several counties, will not exceed a previously assigned maximum.

THE GENERAL PROBLEM AND THE DATA

Fundamentally the problem is one of sampling. Its object, stated statistically, is to reduce the standard error of a mean to the practicable minimum. Theoretically this may be done either by eliminating all irrelevant variation from the standard deviation of the whole unit of data, or by increasing the number of variates or measurements, as is evident in the following formula:

$$\sigma_M = \frac{\sigma}{\sqrt{N}} \text{-----} (1)$$

¹ Received for publication Mar. 18, 1932; issued December, 1932.

in which

σ_M = the standard error of the mean

σ = the standard deviation of the universe, and

N = the number of variates or measurements

There are, however, practical difficulties in either of these procedures. On the one hand, the standard deviation is a characteristic of the universe dealt with. It is an aggregate of variation from an indefinite number of causes; and only that portion which is recognized by quantity and by source may be logically deducted, leaving the residual error of estimate. On the other hand, the number of variates that may be collected is limited by time and funds available for the field work of a survey. In order, therefore, to find out what reliability of the mean can be attained with given funds it is necessary to gather a certain amount of data from a given universe and, first of all, analyze the standard deviation in order to arrive at the proper value for σ , at the same time keeping cost account of the field work to establish the highest practicable numerical value of N .

With these ends in view, a preliminary survey was made in the spring of 1931 in East Carroll and West Carroll Parishes in north-eastern Louisiana. These parishes lie wholly within the Mississippi River Delta region² and contain bottom-land hardwood forests typical of all but the southernmost portion of the Delta. The forest and nonforest types are arranged in irregular north and south belts paralleling the Mississippi River and smaller drainages. Immediately west of the Mississippi there is a belt of "batture" land that extends to the levee. It is partly to completely overflowed every year and is almost entirely forested, principally with willow (*Salix* sp.) and southern cottonwood (*Populus deltoides virginiana* (Castigl.) Sudw.). Just west of the levee is a belt of rich agricultural land, planted largely to cotton. Beyond this lie, in order, a belt of cut-over bottom-land hardwoods; a belt of agricultural land along Bayou Macon, which separates the two parishes, and on Macon Ridge; and finally a broad belt of bottom-land hardwoods which contains, in two large tracts, the bulk of the virgin timber. These so-called belts are, of course, by no means entirely of the classification given but are principally as described.

The sample survey was made by what is known as the line-plot method. Ten parallel survey lines, 3 miles apart, were run due east and west in order to cross the prevailing drainages approximately at right angles. At each 10-chain (660 feet) interval along the lines, a quarter-acre sample plot was established on which, if in the forest, measurements were made; if not, the nonforest type was noted. In this way, 240 miles of line were measured, and 1,918 plots were taken, of which 1,189 fell within the forest. Since the total area of the two parishes by the most reliable estimate is 503,000 acres, nearly 0.1 per cent of the area is actually in sample plots. Without entering into any detailed account of the manner in which the data were collected on each plot, or the exact nature of the many items of information, it is proposed to analyze the manner and extent of sampling to determine its adequacy with respect both to land area by forest and

² The Mississippi River Delta region extends from above Cape Girardeau, Mo., some 60 miles northwest of the mouth of the Ohio River, to the Gulf of Mexico, and comprises an area of about 29,000,000 acres.

nonforest classes and to volume of timber by species and forest-condition class.

AREA ANALYSIS

The object of the area analysis is to arrive at a correct and feasible method of estimating the land area in the following land classes, together with a statement of the error of estimate of each: Total forest land; forest land in each of 5 forest sites—ridge, flat, swamp, batture, and upland;³ forest land in each of 7 forest conditions—virgin, culled, cut-over and restocking, cut-over and not restocking, ruined, second growth, and old field;³ total land not in forest (non-forest); and nonforest land in each of 11 nonforest conditions.

There are two sets of data both suitable for analysis. First is the number of plots counted in each land class in terms of the entire number of plots in the survey. Analysis of these data involves homograde statistics or the statistics of attributes. The other possibility is the linear distance traversed in each land class in terms of the entire length of line in the survey. Analysis of these data involves heterograde statistics or the statistics of variables.

TABLE 1.—Summary of land class area data for East Carroll and West Carroll Parishes, La.

Land-area class	Line survey		Plots	
	Chains	Per cent	Number	Per cent
Forest site:				
Ridge	4,708	24.56	469	24.45
Flat	6,198	32.33	616	32.12
Swamp	301	1.57	23	1.20
Batture	637	3.32	65	3.39
Upland	176	.92	16	.83
Total in forest	12,020	62.70	1,189	61.99
Forest condition:				
Virgin	1,515	7.90	154	8.03
Culled	228	1.19	23	1.20
Cut-over restocking	7,654	39.93	758	39.52
Cut-over nonrestocking	1,032	5.38	93	4.85
Ruined	488	2.55	55	2.87
Old field	533	2.78	50	2.60
Second growth	570	2.97	56	2.92
Total in forest	12,020	62.70	1,189	61.99
Nonforest type:				
Cultivated farm land	4,721	24.63	470	24.51
Deadening	1,034	5.39	114	5.94
Pasture land	541	2.82	59	3.08
Wood-lot pasture	317	1.65	33	1.72
Abandoned farm land	143	.75	14	.73
Prairie				
Marsh	26	.14	3	.16
Lake	61	.32	5	.26
Roads, railroads, etc.	138	.72	9	.47
Towns, villages	23	.12	2	.10
Rivers, lakes, etc.	145	.76	20	1.04
Total, nonforest	7,149	37.30	729	38.01
Total area	19,169	100.00	1,918	100.00

When the data are listed according to both methods, as in Table 1, it is evident that, on the basis of percentages of area, the two methods check satisfactorily. If the resulting errors of estimate were also to check, the use of the plot count would be preferred because of the

³ For definitions of these sites and conditions, see the following publication: LENTZ, G. H. THE FOREST SURVEY IN THE BOTTOMLAND HARDWOODS OF THE MISSISSIPPI DELTA. Jour. Forestry 29: 1046-1055. 1931.

simplicity of its application, and because the linear measurement of area classes traversed in the field could then be omitted entirely.

PLOT-COUNT METHOD

The plot-count method is useful in determining whether we are dealing with a true random sample of land-area classes; that is, whether the statistical sample—the number of plots by land-area class—has been selected in such a way that any plot has been as likely of selection from the statistical universe (the bottom-land area as represented by East Carroll and West Carroll Parishes) as any other. If we have such a sample, the standard deviation of the number of plots in a given land class is

$$\sigma = \sqrt{npq} \text{-----} \quad (2)$$

in which

- σ = the standard deviation of the number of plots
- p = the chance that a plot is of a given land class
- q = the chance that it is not, where $p + q = 1$
- n = the total number of plots

The standard error of the proportion of plots in a given land class may be written

$$\sigma_p = \frac{\sigma}{n} = \sqrt{\frac{pq}{n}} \text{-----} \quad (3)$$

This is a more useful measure for the final expression because it gives, directly, the standard error of a particular land class as a percentage of the total land area. For comparison with actual dispersion, however, that measure will be used which is the handier for the particular purpose. In the following application of formula 3, the cut-over restocking forest condition is used because it contains the greatest number of plots. According to Table 1 there are:

- 758 plots in cut-over restocking condition
- 1,160 plots in other conditions (both forest and nonforest)
- 1,918 plots in all

hence

$$p = \frac{758}{1918} = 0.395$$

$$q = \frac{1160}{1918} = 0.605$$

and from equation 2

$$\sigma = \sqrt{1918 \times 0.395 \times 0.605}$$

$$= 21.5 \text{ plots}$$

This means that, provided the conditions of random sampling have been fulfilled, it is to be expected that in 2 out of 3 additional sets of 1,918 plots the number in the cut-over restocking conditions will be within 22 of the present count.

But this is the point to be investigated. We need to know whether the standard deviation calculated in this way from the 1,918 plots is identical with the standard deviation that would be obtained if all the quarter acres of land area in the two parishes could be thrown into an urn, thoroughly mixed, and a number of samples consisting of 1,918 plots each could be drawn out. Obviously such a test can not be made. However, the principle may be tested by means of two different schemes of setting up data for the calculation of the actual standard deviation. The first of these is a system of replications or

mechanical groupings, in which, for example, group 1 is composed of plots Nos. 1, 21, 41, 61, etc., group 2 is composed of plots Nos. 2, 22, 42, 62, etc., making in all 20 groups with about 96 plots in each group. Another scheme compares the relative number of plots in cut-over restocking condition from one survey line to another, making 10 groups, one for each line.

TEST BY MECHANICAL GROUPING

From the distribution of the mechanical groupings as presented in Table 2, the mean and standard deviation are reckoned as follows:

Mean = 37.9 ± 0.62 plots out of 96

Standard deviation = 2.75 ± 0.44 plots out of 96

TABLE 2.—Frequency of plots in cut-over restocking condition in 20 mechanically set up groups of 96 plots each

Group No.	Number of plots of cut-over restocking	Group No.	Number of plots of cut-over restocking
1.....	35	12.....	37
2.....	38	13.....	34
3.....	33	14.....	37
4.....	33	15.....	37
5.....	41	16.....	38
6.....	38	17.....	42
7.....	41	18.....	40
8.....	40	19.....	39
9.....	43	20.....	39
10.....	36		
11.....	37		758

Now, the standard deviation to be expected from a random sample of 96 plots is, from equation 2:

$$\sigma = \sqrt{96 \times \frac{37.9}{96} \times \frac{58.1}{96}} \\ = 4.79$$

It is evident that 2.75 ± 0.44 can not be accepted as an accidental deviation from 4.79. However, familiarity with the region from which these samples are drawn helps to explain how this scheme of grouping plots might result in a subnormal dispersion. Each plot is but one-eighth mile distant from its immediate neighbors on the same line. Since areas of cut-over restocking condition, when encountered in the field at all, are usually extensive, it follows that wherever one plot in this condition occurs a number of consecutive ones are likely to be found. Since each consecutive plot has been put into a consecutive group, the variability from group to group is less than the variability of random samples.

TEST BY SURVEY-LINE GROUPING

Table 3 gives the total number of plots on each line and the number and percentage of cut-over restocking plots on each, without regard to length of line. From these percentages the following calculations are made:

Mean = 39.0 ± 2.42 per cent

Standard deviation = 7.65 ± 1.71 per cent

Average number of plots per line = 192

TABLE 3.—Frequency of plots in cut-over restocking condition, by survey lines

Line No.	All plots	Frequency of cut-over restocking		Line No.	All plots	Frequency of cut-over restocking	
		Number	Per cent			Number	Per cent
1.....	115	46	40	7.....	260	118	45
2.....	150	53	35	8.....	210	96	46
3.....	201	71	35	9.....	209	117	56
4.....	197	72	37	10.....	171	57	33
5.....	200	71	35	Total.....	1,918	758	-----
6.....	205	57	28				

However, the theoretical standard error of the proportion is, by equation 3:

$$\sigma_p = \sqrt{\frac{0.395 \times 0.605}{192}} \\ = 0.0353 \text{ per plot or } 3.53 \text{ per cent}$$

The abnormal variation of the areas from line to line as compared with the theoretical dispersion demonstrates that the areas of cut-over restocking lands are by no means evenly distributed over the two parishes; that there is, rather, a relationship between percentage of cut-over restocking area and "place" in the two parishes.

It may be argued that since the actual variation may be made subnormal or abnormal depending upon the method of grouping, the theoretical standard deviation of the ungrouped data—21.5 plots out of the 1,918—may be a sufficiently satisfactory one, in that it should average out the high and low dispersions. The fallacy of this will be brought out in the test problem to be discussed later.

LINEAR-MEASUREMENT METHOD

The linear-measurement method differs from the plot count in one important respect. Whereas the plot count of a land-area class is merely alternative, in that a given plot either is or is not of the class to be investigated, the linear measurement is qualitative, in that the distance traversed in the land class under investigation is measured. The problem, then, becomes one of analysis of variation of measurement on the hypothesis that the area of a given land class varies as the linear distance traversed within it. A discussion of this hypothesis will be taken up later.

To begin with, it is necessary to determine which unit length of line (e. g., one-half mile, 3 miles) gives the most stable measure of the dispersion of a land-area class. In this determination the cut-over restocking forest condition is again used.

TESTS BY CONTINUOUS UNITS

The lengths analyzed are the ½-mile, 3-mile, and 24-mile units; for each of these the standard deviation and the standard error of the mean in number of chains are calculated and reduced for ready comparison to number of chains to the half mile. (Table 4.)

TABLE 4.—*Comparison of standard deviations and standard errors of the mean for various unit lengths for survey line in cut-over restocking condition*

Length of unit	All units	Cut-over restocking statistics per one-half mile		
		Mean	Standard deviation	Standard error of the mean
	<i>Number</i>	<i>Chains</i>	<i>Chains</i>	<i>Chains</i>
$\frac{1}{2}$ mile.....	480	16	15.9	0.73
3 miles.....	80	16	8.76	.98
24 miles.....	10	16	4.78	1.51

The increasing standard error of the means for units greater than one-half mile in length, as shown in Table 4, clearly indicates that the variation in the data from the longer units, like the abnormal variation of the plot count when full line units were used, is correlated with "place."

TEST BY RANDOMLY GROUPED $\frac{1}{2}$ -MILE UNITS

Although Table 4 shows that the error of land-class estimate is less with the $\frac{1}{2}$ -mile unit than with the longer unit lengths, the standard deviation—15.9 chains to the half mile—is practically as great as the mean of 16 chains. This follows from the fact that the distribution is U-shaped; of the four hundred and eighty $\frac{1}{2}$ -mile units, 155 contain no chainage at all of cut-over restocking and 88 are entirely cut-over restocking.

On this account the standard deviation is subject to an error greater than that of unimodal distributions; and as the standard error of the mean of any distribution varies directly as the standard deviation, this also is subject to greater fluctuation in this type of distribution. Therefore, rather than to rely upon a single standard deviation from which the error of the mean is to be calculated, actual variations of means from groups or randomly selected $\frac{1}{2}$ -mile units were compared with the theoretical. For this purpose, the number of chains of land in cut-over restocking condition in each of the four hundred and eighty $\frac{1}{2}$ -mile units was tallied upon small, metal-rimmed, circular tags, such as are used for price tags. The 480 tags were thoroughly mixed in a box, drawn out one at a time, and the values noted in the order of draw. Four groupings were thus made. Their standard deviations are given in Table 5.

TABLE 5.—*Comparison of standard deviations of groups of random-selected $\frac{1}{2}$ -mile units in cut-over restocking condition*

Units in group	Groups	Standard deviation per one-half mile	Units in group	Groups	Standard deviation per one-half mile
<i>Number</i>	<i>Number</i>	<i>Chains</i>	<i>Number</i>	<i>Number</i>	<i>Chains</i>
12	40	4.71	24	20	2.61
20	24	3.82	40	12	2.72

Figure 1 summarizes graphically the work thus far on the linear-measurement method. It shows the standard deviation of $\frac{1}{2}$ -mile units and the standard deviation of the 3-mile and 24-mile continuous units, all from Table 4; the standard deviations of distribution of the randomly grouped $\frac{1}{2}$ -mile units, from Table 5; and the curve of the theoretical standard deviation of randomly grouped $\frac{1}{2}$ -mile units. Figure 1 indicates that unit lengths greater than one-half mile introduce systematic errors of the mean, the result of correlation of cut-over restocking areas with "place" in the two parishes. On the other hand, the actual error of the groups, after the effect of "place"

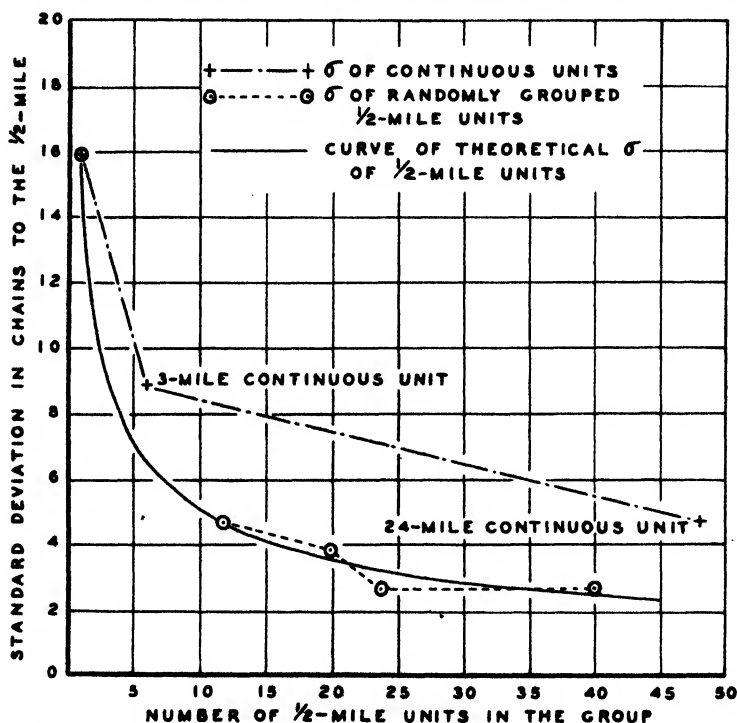


FIGURE 1.—Comparison of the standard deviations resulting from the various methods of grouping with the curve of the theoretical standard deviation. Cut-over restocking condition

is removed by random selection of $\frac{1}{2}$ -mile units, checks very well with the theoretical.

It is concluded, therefore, that when areas in land units similar to these two Louisiana parishes are determined by the linear-measurement method, the error of sampling of a land class may be deduced directly from the distribution of ungrouped $\frac{1}{2}$ -mile units.

PLOT COUNT VERSUS LINEAR MEASUREMENT

Up to this point it has been satisfactorily established (1) that the plot-count method exposes systematic variation, if present, but does not give stable errors of estimate of land-class area; and (2) that if the linear-measurement method is employed, the use of the $\frac{1}{2}$ -mile

unit results in errors of estimate free from systematic variation over the territory sampled. But this gives little indication of which method is to be preferred. It may be, for instance, that the error of the ungrouped plot-count data—the 21.5 plots out of the 1,918, or about 1 per cent of the land area—is sufficiently close to the truth in that it averages the subnormal and abnormal variations of the grouping schemes used. The choice of method rests upon the agreement of estimated areas with the true areas. But such comparisons can not be made for the land classes as defined because we have no true areas upon which to base them.

Recourse was had in this instance to a soil-type map of East Carroll and West Carroll Parishes upon a scale of 1 inch to the mile.⁴ As the different soil types roughly parallel the Mississippi River and interior drainages just as do the forest sites and conditions, this served very well as a medium for the comparison of method, in the following manner:

The 10 parallel east and west survey lines actually determined were drawn on the map, 3 miles apart, establishing as line universes the area within $1\frac{1}{2}$ miles on either side of each survey line.

The area of each soil type was planimetered by line universe. Seven soil types occurred on nine of the line universes and three soil types on the remaining one. The planimetered areas of these 66 cases were taken as the true areas of the soil types, the base for comparison of sampling method.

The sampling process on the map resembled the sampling of land-area classes in the field—(1) by counting plots at every 10 chains along the lines and noting the soil type of each, and (2) by scaling the distance in chains across each soil type traversed by the survey lines.

TEST OF THE PLOT-COUNT METHOD

The difference between the actual areas and the areas estimated by plot count were calculated in terms of the standard error of the latter for each of the 66 cases. A frequency distribution of these ratios—if the method has fulfilled the requirement of random sampling—should have a standard deviation of 1, and its departure from 1 may be considered a measure of the failure of the sampling method. The following example of Sharkey clay soil type on line universe 1 serves as an illustration:

Planimetered area of Sharkey clay = 3,456 acres

Planimetered area of line universe = 26,132 acres

Relative area of Sharkey clay = $\frac{3456}{26132} = 0.132$

Number of Sharkey clay plots on line 1 = 18

Total number of plots on line 1 = 112

Relative number of Sharkey clay plots = $\frac{18}{112} = 0.161$

Standard error of relative number of Sharkey clay plots:

$$\sigma_p = \sqrt{\frac{0.161 \times 0.839}{112}} = 0.035$$

⁴ WORTHEN, E. L., and BELDEN, H. L. SOIL SURVEY OF EAST CARROLL AND WEST CARROLL PARISHES, LOUISIANA. U. S. Dept. Agr., Bur. Soils Field Oper. 1908, Rpt. 10: 875-898, illus. 1911.

Difference between actual and estimated areas in terms of the standard error of the latter is

$$\frac{0.132 - 0.161}{0.035} = -0.8\sigma$$

The distribution of the ratios is shown in Table 6.

TABLE 6.—*Distribution of the ratios of actual errors of soil-type area to estimated errors by the plot-count method*

Error ^a	Fre- quency	Error ^a	Fre- quency	Error ^a	Fre- quency	Error ^a	Fre- quency
+5.5	-----	+2.0	6	-1.5	5	-4.5	-----
+5.0	1	+1.5	5	-2.0	2	-5.0	-----
+4.5	1	+1.0	6	-2.5	1	-5.5	1
+4.0	-----	+0.5	6	-3.0	2		
+3.5	-----	0	8	-3.5	-----		66
+3.0	3	-0.5	11	-4.0	-----		
+2.5	1	-1.0	7				

^a The difference between the actual area and the estimated area in terms of the standard error of the estimated area.

Their standard deviation is

$$\sigma = 1.72 \pm 0.149$$

Had we assumed the sampling method to have fulfilled the requirements of random sampling we would have taken for granted that the standard error of the plot count would give the range within which the true error lies two times out of three, or with odds of 2 to 1. We find, however, that the actual error is, most probably, 72 per cent higher; that the estimated standard error is in reality $\frac{1}{1.72}$, which, by reference to the normal curve of error, is equivalent to odds of but 0.8 to 1.

TEST OF THE LINEAR-MEASUREMENT METHOD

In testing the linear-measurement method essentially the same kind of comparison was made as in the case of the plot-count method. The following is an illustration of the calculation in one case, employing the Sharkey clay on line universe 1:

Relative area of Sharkey clay = 0.132 (as above).

Statistics from frequency distribution of linear measurements, shown in Table 7:

TABLE 7.—*Frequency distribution of scaled distances of Sharkey clay land on line 1*

Chains of Sharkey clay to the one-half mile	$\frac{1}{2}$ -mile units	Chains of Sharkey clay to the one-half mile	$\frac{1}{2}$ -mile units	Chains of Sharkey clay to the one-half mile	$\frac{1}{2}$ -mile units	Chains of Sharkey clay to the one-half mile	$\frac{1}{2}$ -mile units
	Number		Number		Number		Number
0	23	15	-----	30	-----	40	3
5	-----	20	2	35	-----		
10	1	25	-----			Total....	29

Mean = 5.17 chains to the half mile

Standard deviation = 10.7 chains to the half mile

Standard error of mean = 1.99 chains to the half mile

Hence, relative area of Sharkey clay = $\frac{5.17}{40} = 0.129$

Standard error or relative area of Sharkey clay:

$$\sigma_p = \frac{1.99}{40} = 0.050$$

Difference between actual and estimated areas in terms of the standard error of the latter:

$$\frac{0.136 - 0.129}{0.050} = +0.1\sigma$$

The distribution of the ratios are shown in Table 8.

TABLE 8.—*Distribution of the ratios of actual errors of soil-type area to estimated errors by the linear-measurement method*

Error ^a	Fre- quency	Error ^a	Fre- quency	Error ^a	Fre- quency	Error ^a	Fre- quency
+4.2	1	+2.1	---	0	9	-2.1	---
+3.9	---	+1.8	3	-.3	15	-2.4	---
+3.6	---	+1.5	1	-.6	4	-2.7	---
+3.3	1	+1.2	2	-.9	2	-3.0	1
+3.0	---	+.9	7	-1.2	4	---	---
+2.7	---	+.6	8	-1.5	---	---	66
+2.4	---	+.3	7	-1.8	1	---	---

^a The difference between the actual area and the estimated area in terms of the standard error of the estimated area.

The standard deviation is:

$$\sigma = 1.05 \pm 0.093$$

This does not differ significantly from 1 and therefore satisfies the requirement that the estimated errors be the true errors of estimate. Furthermore, since no correlation is found between the ratios and the size of area estimated, it is proved that the area of a land class, in a universe represented by these two parishes, varies as the linear distance traversed within it.⁵

COMPARISON OF METHODS

The final results of the two sampling methods are compared in Table 9, the last three columns of which show the errors of estimate. On the assumption that plot counts at spacings of 10 chains by 3 miles satisfy the conditions of random sampling, the resultant error by this method is the lesser. As has been brought out, however, this assumption is erroneous, as it requires, for the soil-type data at hand, a correction factor of 1.72. This correction has been applied in the table, the two right-hand columns bringing out the properly comparable errors.

⁵ At first thought it seemed more logical to one of the authors that the area of a land class should vary as the sums of the squares of the linear distances traversed within it. Walter H. Meyer, of the Pacific Northwest Forest Experiment Station, tested this hypothesis and found that it leads to estimates that are too high whenever a single land class occurs as an extensive, unbroken area traversed by two or more survey lines.

TABLE 9.—Relative areas of soil types and comparison of their standard errors of estimate by different sampling methods

Soil type ^a	Relative areas of soil types			Standard error of area as a percentage of corresponding area		
	By planimeter	By plot count	By linear measurement	By plot count		By linear measurement
				Random sampling	Corrected for sampling ^b	
	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
YS.....	5.5	5.3	4.2	10.0	17.2	24.0
YL.....	6.4	5.8	6.5	9.5	16.3	15.0
WC.....	17.2	19.6	18.1	4.7	8.1	9.3
SC.....	25.6	24.2	24.5	4.1	7.1	7.9
WSL.....	8.9	9.8	9.9	7.1	12.2	14.1
RL.....	17.8	17.3	18.7	5.1	8.8	9.0
CS.....	18.3	18.0	16.6	5.0	8.6	9.8
Total.....	c 99.7	100.0	d 98.5			

^a Symbols for soil types are those given on the map mentioned in text.

^b This correction is 1.72 times the value obtained on hypothesis of random sampling.

^c Three-tenths of 1 per cent of the total area is made up of 3 soil types which are not included because they are not encountered in the plot-count method.

^d The 3 soil types not given in the table account for the remaining 1.5 per cent.

In six of the seven soil types, the corrected error of the plot-count method is still less than the error of the linear-measurement method. It is very doubtful, however, whether the differences in its favor are to be considered as a recommendation, for the correction is an empirical one, and may safely be applied only to this soil-type test. The error of linear measurement, on the other hand, is the outcome of variation actually encountered; it calls for no correction, because by comparison with the universe from which the data were drawn, it satisfies the limitations of random sampling.

VOLUME ANALYSIS

The volume data consist of the board-foot contents, by species, of the timber on 1,208 sample plots ^a taken in the forest. From these the following values for all species together have been calculated:

Mean = 659 board feet

Standard deviation = 854 board feet

Standard error of mean = 24.5 board feet

But the standard error of the mean varies directly as the standard deviation of the distribution, and the latter measure is an aggregate of variation from many causes. One of these readily recognized in the field is the forest condition.

The plots were accordingly sorted by condition. The mean volume and standard deviation of each are as given in Table 10. The standard deviations are, in fact, standard errors of estimate of plot volume free from the influence of forest condition.

^a This does not agree with the 1,189 forest plots of Table 1. The field instructions called for the measurement of a forest plot whenever a nonforest plot happened to fall within 2 chains of the forest, the purpose being simply to strengthen volume data. The added 19 plots were not used in area analyses.

TABLE 10.—Means and standard deviations of plot volumes by forest condition

Forest condition	Basis, plots	Mean volume per plot	Standard deviation of volume per plot
	<i>Number</i>	<i>Board feet</i>	<i>Board feet</i>
Virgin.....	155	1,886	1,232
Culled.....	22	1,779	792
Cut-over restocking.....	761	478	533
Cut-over not restocking.....	100	354	576
Ruined.....	59	96	200
Old field.....	55	360	450
Second growth.....	56	700	976

The average of these, weighted by number of plots, may be expressed:

$$\bar{\sigma} = \sqrt{\frac{N_1\sigma_1^2 + N_2\sigma_2^2 + \dots + N_n\sigma_n^2}{N_1 + N_2 + \dots + N_n}} \quad (4)$$

in which

$\bar{\sigma}$ = the weighted mean value of standard deviations from n frequency distributions

σ = the standard deviation of each distribution

N = the number of plots in each distribution

and subscripts 1, 2, . . . n refer to the individual distributions.

The weighted mean value of the standard deviations of Table 10 thus becomes 684 board feet.⁷ Hence, by equation 1, the standard error of the mean, free from the influence of forest condition, becomes 19.7 board feet.

The data were further scrutinized for evidence of relationship between the remaining variation of the mean and other factors, particularly site and locality, but nothing of significance was found.⁸ Hence it is concluded that 19.7 board feet to the quarter acre is the error of sampling. This is 2.99 per cent of the mean volume.

The error of the total volume of the two parishes, however, must be greater than 2.99 per cent because it is affected not only by volume variation from plot to plot, but also by the error of estimate of total forest area. In short, the correct estimate of volume on a large forest area is

$$(A \pm \sigma_A) (M \pm \sigma_M)$$

in which

A = the total forest area, in this case in quarter acres

M = the mean volume to the quarter acre

σ_A = the standard error of the total forest area

σ_M = the standard error of the mean volume

This equation has been shown to be:

$$AM \pm \sqrt{A^2\sigma_M^2 + M^2\sigma_A^2} \quad (5)$$

⁷ We may calculate a measure of the effect of forest condition upon the volume of hardwoods in the two parishes. For the squared variation of volume free from the influence of condition, $[(.084)^2] +$ the squared variation of volume associated with condition = the total squared variation $[(.854)^2]$. From this equality we find that 35.8 per cent of the total squared variation of volume is associated with forest condition. Hence the correlation ratio, which is calculated directly from the square root of this value, is 0.598 ± 0.018 .

⁸ A standard deviation below the 684 board feet obtained by stratifying according to condition may be arrived at by grouping the plots either systematically or at random and finding the standard deviation of the means of the groups. This value times the square root of the number of plots per group gives the residual standard deviation of plot distribution. With 20 or less groups a standard deviation of 500 board feet has been obtained. However, there is no justification for the reduction as it is impossible to state just what factor or factors of correlated variation have been averaged out in the process of grouping.

The best estimate of the total area of the two parishes is 503,000 acres,⁹ and this is taken as without error. The total forest area, calculated by the linear-measurement method, is 62.7 ± 2.95 per cent of this, or $1,261,524 \pm 37,215$ quarter acres. Since the mean volume to the quarter acre is 659 ± 19.7 board feet, equation 5 gives a total volume in the two parishes of 831 ± 34.9 million board feet and the standard error is 4.20 per cent of the total.

We now have a working basis for calculating the total number of miles of survey line that must be run across land units similar to East Carroll and West Carroll Parishes in order to determine the total volume of merchantable timber within any given limit of accuracy. The total number of miles, with a plot taken every 10 chains, is one-eighth of the total number of plots and, in the present case, almost exactly one-fifth of the number of forest plots (for forest area equals 62.7 per cent of the total area). From equation 1 we have

$$N = \left(\frac{\sigma}{\sigma_M} \right)^2 \text{-----} \quad (6)$$

where

N = the number of forest plots

The numerator to use for ready calculation of equation 6 should be that standard deviation which measures variation of plot volume and forest area, but which is free from the influence of variation in forest condition. We have this measure, but it is expressed as the standard error of the mean (or total)—the 4.20 per cent arrived at above. The corresponding standard deviation is $\sqrt{1,208}$ plots times this value. The denominator should be the standard error expressed as a percentage of the mean (or total)—that is, the measure of precision desired.

The results in number of plots or number of miles of line must be coordinated with the results similarly obtained from other analyses, such as volume of certain important species or volume by log scale, before any final estimate of the amount of data needed can be rendered.

COORDINATION AND APPLICATION OF ANALYSES

In conclusion, it remains only to show the practical use or application of the calculated errors of sampling and the way in which the errors for the different items of area and volume are coordinated. The vital question, indicating the *raison d'être* for the foregoing analyses, is: How many miles of survey lines must be run to obtain acceptable limits of error in all important phases of the survey? From the number of miles, the distance between lines is of course readily calculated when the area of the land unit to be surveyed is approximately known.

To answer this question, it is necessary first to coordinate the different errors for the same mileage of line by putting them in such form that they can readily be compared. This is conveniently done by making a table that shows the standard error (in percentage of

⁹ This figure has been arrived at after comparing estimates of the area concerned as established by the Bureau of Chemistry and Soils and the Bureau of Agricultural Economics, United States Department of Agriculture, and the county surveyor.

the mean) for each important item for several selected mileages of line. The mileages selected should be within the range of practical possibility as determined by the size of the land unit to be surveyed, the cost per mile of line, the funds available, the approximate permissible errors, etc. Table 11 is a sample portion of such a tabulation.

TABLE 11.—*Standard errors of important items for different total lengths of line*

[A sample portion of a tabulation from which the required mileage of survey lines can most easily be determined. Eight plots taken per mile. Each standard error expressed as a percentage of the mean of the item concerned]

Item to be evaluated	Percentage of the mean for the indicated total number of miles of line run				
	200	300	500	1,000	1,200
	At standard error of --				
	$x \sqrt{5}$	$x \sqrt{3.33}$	$x \sqrt{2}$	x	$\frac{x}{\sqrt{1.2}}$
Area:					
Virgin forest	15.4	12.6	9.8	6.9	6.3
Cut-over-restocking forest	4.7	3.8	3.0	2.1	1.9
Total forest	3.2	2.6	2.0	1.4	1.3
Volume (including error of area):					
Virgin forest	16.6	13.5	10.5	7.4	6.7
Cut-over-restocking forest	6.5	5.3	4.1	2.9	2.6
Red gum	12.3	10.1	7.8	5.5	5.0
Total forest	4.5	3.6	2.8	2.0	1.8
(Etc.)					

The greatest acceptable error in the most important item (in this case, and usually, total merchantable volume) may then be decided upon and the corresponding mileage read directly or interpolated from the table. The errors in other items for this mileage are then noted and if any of them are unsatisfactory the mileage may have to be increased. Many compromises and concessions will invariably be necessary before the errors in most or all of the items are declared satisfactory for a financially practicable mileage.

In the bottom-land hardwood region the forest survey proper will be started in a land unit of about 5,500 square miles in northern Louisiana. After a detailed study of a tabulation of expected errors for given mileages, of which Table 11 is a small part, it is estimated that the results for this unit will be sufficiently close to the truth if the total mileage run is 550. This gives a line interval of 10 miles. The standard errors expected for the several items of Table 11 are about 95 per cent of those tabulated therein in the 500-mile column.

SUMMARY

Samples of land-area measurements and timber-volume data were taken in two parishes in northeastern Louisiana by the line-plot system, for the purpose of determining the probable errors of estimate by land-area classifications and timber volumes to be expected from a projected forest survey of similar nature of 29,000,000 acres in the bottom-land forest region.

The land-area analyses disclosed that the number of plots in the various land classes, while correctly establishing their proportional areas, can not be considered as random samples of the land classes.

This is because neither the standard deviation as calculated from 20 mechanically set-up groups nor the standard deviation as calculated from the variation between the survey lines, checked with the standard deviation to be expected upon the hypothesis of random sampling.

The linear-measurement method of area by land classification was analyzed as an alternative to the plot-count method. The error of estimate was tested by using unit lengths of one-half mile, 3 miles, and 24 miles, and was found to increase with unit lengths longer than the one-half mile.

The comparison of the errors of estimate as between the plot-count and the linear-measurement methods was based upon a test problem from the soil-type map of the two parishes. The true areas in each soil type were arrived at by planimetering the map. The soil types were then sampled on the map in a manner analogous to that by which land-area classes were sampled in the field. The comparison of sampling method is based upon the agreement of the actual with the estimated sampling errors. It was found that the actual errors of the linear-measurement method agreed with the estimated errors.

In the volume analyses, the standard error of the mean volume in board feet was found to be about 3 per cent of the mean volume per $\frac{1}{4}$ -acre plot; and this value is the residual after taking out that portion of the error which is due to variation in forest-condition classes. Since the total volume in the forests of the two parishes is the product of the mean volume per $\frac{1}{4}$ -acre plot and the number of quarter acres in forest area, with their respective errors of estimate, the standard error of estimate of the total volume becomes slightly higher than 4 per cent of the total volume.

The results of the area and volume analyses were used as the basis for the recommendation that in the forest survey proper the line interval be 10 miles.

DEFINITIONS OF HONEY COLOR GRADES ¹

By E. F. PHILLIPS

Professor of Apiculture, New York (Cornell) Agricultural Experiment Station

INTRODUCTION

Grading according to color has long been an important factor in the marketing of honey. In different countries and by different organizations of beekeepers and honey dealers, various methods have been employed for evaluating and describing the colors of honey. In some instances names of color grades have been used without any attempt to define the grades or to employ standard colors for grading. In other cases colored glasses or bottles of colored liquids (often quite different in color from honeys) have been used. Obviously it is desirable that some accurate method be devised for defining and measuring the color grades of honey and that the same standards be widely used.

IMPORTANCE OF COLOR GRADING

The most important reason for grading honey by color is that flavors of honeys from any given plant source vary with color, as certain examples will show. In the irrigated regions of the western part of the United States alfalfa honey is produced, usually with an admixture of honey from sweetclover but with less than the customary admixtures of nectars from other plant sources. In those parts of the irrigated region in which the altitude is high the honey from alfalfa and sweetclover is the most delicate and mild obtainable from these plants. In the Imperial Valley, lying below sea level, the honey from these plants is a rather dark amber, and the flavor is stronger and less delicate. It was formerly supposed that these differences were caused by admixtures of other nectars, but it is now known that they are due to the effect of climate, soil, and altitude on the color of the nectar of the plants. If, therefore, a buyer of honey desires either strong or mild alfalfa-sweetclover honey, it is most convenient to designate the color desired, since flavor varies with color.

Similar differences are found in many other honeys. Honey from white and alsike clovers varies from Water White to rather Dark (reddish) Amber, and the flavors differ much as do those of alfalfa-sweetclover honeys, the lighter honeys having the more delicate flavor. For any given plant source, the lightest and mildest honeys usually come from areas near the northern limits of the secretion of nectar from that plant.

Received for publication Jan. 15, 1932; issued December, 1932. The work here reported was carried out during the summers of 1922, 1923, and 1924, while the author was connected with the Bureau of Entomology, U. S. Department of Agriculture, and determinations of light transmissions were made at that time. Later computations were made at the New York State College of Agriculture. A considerable number of persons assisted in the early work. The author is responsible for the final calculations, for the conclusions reached, and for the recommendations of definitions for the honey color grades. The Bureau of Entomology and Agricultural Economics are responsible for the grades recommended in earlier publications, in the devising of which the author took no part.

With certain exceptions mentioned later, it may be said that in general for any given plant origin of honey, the lighter the honey the more delicate the flavor. It thus becomes important to designate the color of honey, for the color designation is of more value in indicating flavor than the name of the plant or plants from which the nectar is derived. Since there is no way of describing flavors as such, some progress at least will be made toward classification by flavors when accurate color grading is made possible. Color grading is, therefore, not a subordination of flavors but is the most accurate way of designating flavors thus far devised.

There are certain honeys for which the demands of the market can not be met on the basis of color grading. Chief among these in the United States is buckwheat (*Fagopyrum esculentum*) honey. This honey is dark and strong in flavor. Within the limits of the United States there is considerable variation in buckwheat honeys. In southern New York and northern Pennsylvania, buckwheat honey is opaque and dark with a slightly purple cast, while in certain areas of the Mid-West this honey is more transparent and is dark amber in color, red and yellow tones predominating. There is a heavy demand for buckwheat honey by the Jewish trade, chiefly in the eastern cities, and for export; for both trades the demand is for the opaque, dark, purplish honey. It is also required that the honey be granulated with a fine grain, even though for certain purposes for which this honey is used, it is liquefied. The flavors of different buckwheat honeys vary, although all of them are strong; it is the flavor of the opaque eastern honey that is preferred in the markets mentioned. Obviously it is impossible to give a color designation that will properly grade buckwheat honey, especially since the honey must be granulated to meet the demands of the market.

Similar conditions seem to prevail with the heather honey of Europe and perhaps also with the honeydew honeys of central Europe. However, the fact that color grading is not feasible in all instances does not make it any less valuable for grading the majority of honeys.

PREVIOUS WORK

The United States Department of Agriculture has indorsed a color grader for honey (Sechrist ² and Sechrist and Samson ³) which is used in grading all honeys intended for export and to some extent those intended for domestic markets. While this grader has largely superseded others and has brought about greater uniformity in color grading, the instrument is so expensive that it is impractical for most beekeepers to buy it.

Any grading standard should be of such a nature that the different grades can be defined in words and figures. It should not depend on comparisons with some manufactured article, on the use of a piece of apparatus manufactured by one firm, or on the experience and skill

² SECHRIST, E. L. THE COLOR GRADING OF HONEY. U. S. Dept. Agr. Circ. 364, 8 p., illus. 1925.

³ ——— and SAMSON, H. W. UNITED STATES STANDARDS FOR HONEY RECOMMENDED BY THE UNITED STATES DEPARTMENT OF AGRICULTURE... U. S. Dept. Agr. Circ. 410, 32 p., illus. 1927.

——— and SAMSON, H. W. UNITED STATES GRADES, COLOR STANDARDS, AND PACKING REQUIREMENTS FOR HONEY. U. S. Dept. Agr. Circ. 24, 32 p., illus. 1927.

of a limited number of individuals. The standard honey grader indorsed by the Department of Agriculture is not based on suitable definitions. For the present deficiencies of definition the writer is chiefly responsible, as appears from the following paragraphs. In the published discussion of this grader ⁴ the percentages of lights of various colors transmitted through 1 cm of honey are given for the darker limits of the several color grades established, from which it might be assumed that these color grades are adequately defined. Such, however, is not the case, for (1) the figures published fail to correspond with the light transmissions measured for the honeys on the darker limits of the several grades, and (2) the official instrument is not based on the published figures.

The foregoing criticism of the definitions of the Department of Agriculture grades calls for some explanation. Before leaving the service of the Bureau of Entomology, the present writer undertook, with the help of his associates in this work, to draw up a table giving the approximate lower limits of light transmissions of three selected wave lengths, as a guide in the establishment of color grades. At the time this table was made it was thought that the limits indicated were fairly satisfactory, and at least moderately accurate. Accordingly, when Sechrist and Samson prepared their circular, they included this table without change except for the omission of figures for two secondary grades. A reexamination of the data for the various honeys studied now discloses the fact that these figures are entirely unsatisfactory, and as definitions for the darker limits of the several grades are practically valueless.

The inadequacy of these figures is indicated by the data shown in Table 1. This table gives the minimum transmission of light for each wave length just as it was prepared by the author and published in part by Sechrist and Samson. After each of these supposedly definitive figures is shown the number of instances among the 290 honeys studied in which the light transmission for the wave length in question is lower than the minimum set by the definition. The total of such cases (two or even more may occur in a single honey) is 269, which is entirely too high a percentage of error to give any validity to the assumed minimum limits. In general, the trends are such as are found in honeys of the several grades, but the actual figures are inaccurate for the purpose for which they were used.⁵ It is therefore clear that so far there are no satisfactory definitions of color grades for honeys. The purpose of this paper is to propose such definitions, based on measurements of light transmission through honeys of a wide variety of colors. Aside from the one attempt mentioned, there appears to be no previous publication that defines color grades for honey.

⁴ — and SAMSON, H. W. *Op. cit.* (Circ. 24, p. 29).

⁵ The author regrets the necessity of pointing out errors in papers by his former colleagues, but since he is chiefly responsible for the original errors, the blame may be placed where it belongs. Sechrist and Samson are in no way responsible for these figures.

TABLE 1.—*Examination of definitions of honey color grades adopted by the United States Department of Agriculture on a basis of minimum transmission of blue, yellow, and red light, showing the number of cases in which the definitions are defective in the 290 honeys studied*

Grade	Blue light		Yellow-green light		Red light	
	Minimum light transmission in definition	Samples below minimum	Minimum light transmission in definition	Samples below minimum	Minimum light transmission in definition	Samples below minimum
	<i>Per cent</i>	<i>Number</i>	<i>Per cent</i>	<i>Number</i>	<i>Per cent</i>	<i>Number</i>
Water White.....	64	8	77	14	81.5	13
Extra White.....	60	9	76	12	80.5	11
White.....	50	19	71	20	78	21
Extra Light Amber.....	48	4	70	4	77	4
Light Amber.....	54.5	15	63	23	73	23
Extra Amber *.....	29	17	60.5	22	70.5	30
Amber.....	15	17	49	22	63	30
Dark Amber *.....	6	—	43	—	59	—

* The grades Extra Amber and Dark Amber are not included in the U. S. Dept. Agr. Circ. 24, table, p. 29. Exceptions to definitions for these grades must here be included in the primary grades, since the extra grades are not clearly indicated in the series of 290 honeys.

MATERIAL AND METHODS

The determinations of light transmission through honeys, here recorded were made during the summer of 1922. Previous to and during that summer, beekeepers in all parts of the country were requested to send samples of honey for this study, and each beekeeper was asked to state the chief nectar source or sources from which the honey was derived. Four hundred and fifty-five samples were received and tasted in the laboratory, and the opinions of the producers regarding the sources of the honeys were found to be quite accurate. From all the honeys received, 290 were finally selected for detailed study.

The honeys selected ranged from the lightest produced in the United States to those so dark that practically no light passed through a stratum 1 cm thick. Most of the honeys were of floral origin, but a few of the darker samples doubtless contained some honeydew honey.

In the measurement of light absorption by liquids, several methods are available. One commonly used is to determine the extinction coefficient; that is, the reciprocal of the depth of solution required to reduce the intensity of light of a given wave length to a definite percentage of that of the entering light. Another method sometimes employed is to measure the energy of the light passing through a liquid of definite thickness and compare it with the energy of the light entering it. Measurements of this type were made by Kutzner⁶ for a few German honeys, but this method, while perhaps the most exact, presents difficulties and unnecessary complications. The method employed in the present work was to measure, by means of a spectrophotometer, the percentage of light of selected wave lengths passing through a stratum of honey 1 cm thick.

Kutzner claims that this method is accurate only to within 3 or 4 per cent, as compared with the light-energy method, but there are other complications, such as turbidity, which complicate all measure-

⁶ KUTZNER, W. ZUR PHYSIK DER HONIGFARBEN. Arch. Bienenk. 9: 185-195, illus. 1928.

ments and which appear to make the method adopted for this work as good as could be chosen. Perhaps the greatest source of error arises from the fact that when the percentage of light passing through a 1-cm stratum of honey is determined by means of the spectrophotometer, the human eye is used to make comparisons of intensities, and this introduces an error which sometimes may be considerable. While determinations were made as accurately as possible, it is useless to assume extreme accuracy, and no such accuracy is necessary, for the honeys of the selected series could often be transposed without material difference in the appearance of the series.

In the early stages of the work, measurements of light transmissions were made for a considerable number of wave lengths, but it was found that there are no regions of the visible spectrum in which unusual absorption occurs (absorption bands), and that when a large number of determinations are made, they form a smooth curve. For practical purposes, therefore, it was found to be entirely satisfactory to confine the routine determinations to three wave lengths, namely, 480, 580, and 680 m μ , these wave lengths being those of the colors blue, yellow-green, and red.⁷

Since the chief purpose of this work was to establish definable color grades for honey, the selected honeys were arranged in series according to their appearance to the eye, from the lightest to the darkest. All the honeys were carefully liquefied to prevent differences due to the deflection of light by included crystals, care being taken not to darken the honeys by overheating.

It is probably true that the proportion of honeys of different color grades in this series, as finally adopted, does not represent the proportion of these grades as they occur in the markets, but such agreement is not necessary. The samples were obtained from all parts of the United States and a majority of them are honeys found in the main honey markets. In the lighter ranges of the series, the gradations were so fine that usually any two adjacent samples might have been transposed without the change being noticed, but in the darker honeys such a transposition would usually have been easily detectable. While the gradations are finer in these honeys, this is permissible because it is chiefly in the lighter honeys that the closer color distinctions are now demanded in the trade.⁸ It should also be noted that in the darker honeys of the series the gradations are less clear and the measurements of light transmissions are more erratic. This was due at least in part to the fact that turbidity is usually more pronounced in darker honeys, but it may also have been partly due to the fact that the darker end of the series was less complete than the lighter.

After the honeys had been arranged in order, some of the most experienced honey graders in the country were asked to indicate where in the series the several color grades should begin and end. Everyone found this a difficult matter to determine, especially in the

⁷ The measurements of light transmission were made by Bernard Kurrelmeyer, who worked on this problem for two summers. The third summer the work was continued by E. W. Tschudi. Throughout all this work, Prof. A. H. Pfund, of the Johns Hopkins University, freely gave advice and assistance. The arrangement of the 200 honeys in color series was the result of cooperation of the entire staff of the bee culture laboratory, as well as of several experienced honey dealers.

⁸ On a uniformly ascending scale of color in the Department of Agriculture honey color grader, the total length of the scale being 14 cm, the following distances are assigned to the various color grades: Water White, 0.8 cm; Extra White, 0.9; White, 1.7; Extra Light Amber, 1.6; Light Amber, 3.5; Amber, 2.9; Dark, 2.6. There are many honeys darker than the darkest part of this scale, so that the actual range of dark honeys is much greater than here indicated. The inequality in length of the different grades is explained by the need for closer color distinctions in the lighter honeys.

lighter honeys. In order that personal prejudice might play no part in the decisions, the floral sources of the honeys were not revealed while the judgments were being passed. To obtain different sets of opinions, the honeys were exhibited at the February, 1923, meeting of the American Honey Producers' League and later at a meeting of beekeepers in Wisconsin. At the meeting of the American Honey Producers' League, a decision as to the limits of the several grades was reached by practically unanimous agreement, separate opinions being taken of a selected set of experienced honey dealers and of all other beekeepers in attendance. These decisions of course referred only to numbers in the exhibited series and had no relation to the actual definitions of the grades.

Previous to this work, different undefined names had been used in the trade for color grades of honeys. The question as to the best designations for the different color grades was discussed at the meeting of the American Honey Producers' League in 1923 and it was decided to retain the names in most common use for the primary color grades.

During this work certain beekeepers insisted that the color grades in common use, especially those for the lighter honeys, were too comprehensive, and that finer subdivisions would be helpful. It appears to be a common experience in the color grading of commodities that with continued use finer and finer subdivisions are demanded. In establishing a grading system, it therefore seemed desirable to make provision for later subdivisions as they might be needed. For such subdivisions of the primary color grades, the additional word "extra" was suggested (except for the lighter subdivision of the dark grade, which is Dark Amber), it being the intention to include in the extra grade about 30 per cent of the lightest part of the primary grade. Thus the grade Extra Light Amber would include about 30 per cent of the lightest honeys of the primary grade Light Amber. The opinion of the honey handlers present at the meeting of the American Honey Producers' League was asked regarding the limits of these, extra grades as well as of the primary grades. The proposed limits were so favorably received that all of them were adopted, except those for the Extra Light Amber grade, which were later changed.

It should be pointed out that the color grades here proposed, or any that might be adopted, are wholly arbitrary, for there are no natural color groupings in honey. Even for honeys of practically pure floral origin, no natural color grouping can be detected, and, furthermore, honeys are so blended in the hive or during extraction that even if natural color groupings occurred in honeys of pure floral origin, the boundaries would be obliterated in the blends. As a result of these facts, there are often border-line cases in which it is impossible to assign a honey definitely to a certain color grade, but in the usual handling of honeys on the market, these cases are less frequent than might be anticipated.

THE ABSORPTION AND DISPERSAL OF LIGHT BY HONEY

When light passes through honey to the eye, the impression formed through reception of the light stimulus is produced by light of various wave lengths. When white light strikes one side of a jar of honey and penetrates the honey, a considerable proportion of the light is absorbed, but this absorption is not equal for lights of all wave lengths.

The absorbed light does not reach the eye but is changed to some other form of energy, chiefly heat. The light which leaves the honey and reaches the eye is therefore a mixture of lights of various wave lengths, in proportions different from that in which they occur in white light, hence the honey appears to be colored.

If honey were clear, more light would pass through than is usually the case, but honeys contain myriads of minute particles of various sizes which either absorb light or deflect the light rays from their direct course. These particles include masses as large as pollen grains, of which there may be thousands in a pound of honey, or other foreign particles which are accidentally included in honeys. The major part of the deflection of light is caused by the presence in all honeys of matter in a colloidal state. The composition of these honey colloids is not altogether understood, but probably they are chiefly dextrans. Honeys differ widely in the turbidity caused by such colloids, some honeys appearing quite clear and others decidedly turbid. The proportion of light which passes through honey is, then, determined not only by the light-absorbing power of the liquid constituents of honey but also by the suspensions occurring in it. The turbidity of honeys seriously complicates the study of light transmission, and gives rise to difficulty in assigning minimum light transmissions for honeys of the several color grades.

The absorption of light varies of course with the thickness of the jar containing the honey, as does also light dispersion or turbidity. Even a Water White honey shows a distinctly amber color if the jar is of considerable thickness, and it also appears more turbid.

The chief constituents of honey are water and several sugars. As light passes through a solution of such sugars, there is some absorption, but this is negligible so far as giving color to the honeys is concerned. Practically all the liquid ingredients of honeys are colorless, by which is meant that they absorb little or no light and the light absorbed is practically the same for all wave lengths. But in addition to the chief ingredients of honey, there are derived through the nectars certain plant dyes which do not permit light to pass without exhibiting differential light absorption. Since honeys from different plant sources differ in color, and since under varying conditions of climate and soils the honey from any one plant source may vary widely in color, it is clear that the colors of honey are due to materials of plant origin, derived through the nectars.

MEASUREMENT OF LIGHT TRANSMISSION

After the honeys had been arranged in series according to color, the proportion of light of each of the three selected wave lengths that passed through 1 cm of each of the 290 honeys was measured. In case any honey had begun to show crystal formation before the light transmissions were determined, it was again carefully liquefied.

It is not necessary to record all the measurements made on these honeys, but a few examples will be helpful in an understanding of the difficulties arising from an attempt to correlate light transmissions with the colors as detected by the eye. The lightest honey in the series, as determined by the eye, permitted 77.5 per cent of the blue light (480 $m\mu$) to pass through, 85 per cent of the yellow-green light (580 $m\mu$), and 91 per cent of the red light (680 $m\mu$). This was a

honey from black sage (*Romona stachyoides*). These percentages are not, however, the highest for any of the three wave lengths as measured by the spectrophotometer, for an alfalfa honey in the series permitted 80 per cent of the blue light and 96 per cent of the red light to pass. Turbidity in the sage honey caused more of the light to be dispersed, although the sage honey appeared lighter to the eye.

A white-clover honey, classified as White, permitted the following proportions of the light to pass: 480 $m\mu$, 58 per cent; 580 $m\mu$, 74 per cent; 680 $m\mu$, 79 per cent. A comparison of the lightest honey with this white honey from clover shows that the reductions in light transmission are relatively rapid. The darkest honey of the series, of unknown floral origin but probably containing some honeydew honey, allowed the following percentages of light to pass; 480 $m\mu$, 0 per cent; 580 $m\mu$, 0.5 per cent; 680 $m\mu$, 15 per cent.

After the honeys had all been measured for the transmission of the lights of the three wave lengths, it was found that the percentages of transmitted light did not fall into a series comparable to that made according to the color appearance of the honeys. This variation is shown in the data from the first 10 lightest honeys of the series. (Table 2.) All 10 of these honeys were so nearly alike in color that it was difficult to tell one from the other. If the data of the light transmissions of these 10 honeys are plotted as curves, a wide band is formed, for the spread is over a range of about 20 per cent.

TABLE 2.—Percentage of light of different wave lengths passing through the 10 lightest honeys studied

Honey No.	Blue light (480 $m\mu$)	Yellow green light (580 $m\mu$)	Red light (680 $m\mu$)	Floral source
58.....	77.5	85.0	91	Black sage (<i>Romona stachyoides</i>).
101.....	75.0	81.5	87	Sage (<i>Romona</i> spp.).
176.....	70.0	80.0	85	Fireweed (<i>Eupilobium angustifolium</i>).
102.....	67.0	76.0	85	Do.
305.....	63.0	74.0	82	Do.
306.....	61.0	74.0	79	Milkweed (<i>Asclepias</i> sp.)
197.....	80.0	92.5	96	Alfalfa (<i>Medicago sativa</i>).
196.....	70.5	80.0	91	Do.
174.....	75.0	84.0	89	Do.
37.....	64.0	74.0	75	Button sage (<i>Romona</i> sp.).

In order to show the extreme limits of light transmission that may fall within a single market grade, the following data are given from the honeys that fall within the lightest classification, known as Water White. For blue light (480 $m\mu$) the highest transmission was 80 per cent, the lowest 53 per cent, and the average 66.2 per cent; for yellow-green light (580 $m\mu$) the highest was 92.5 per cent, the lowest 76.5 per cent, and the average 77.2 per cent; for red light (680 $m\mu$) the highest was 96 per cent, the lowest 73.5 per cent, and the average 82.7 per cent. Thus it is evident that in honeys which appear similar to the eye, there are considerable differences in the actual transmission of light of different wave lengths. Differences of even greater magnitude are found among darker honeys that are similar in appearance.

The data for honeys that were reported to have come from white clover (*Trifolium repens*) and alsike clover (*T. hybridum*) are shown in Table 3. The numbers in column 1 are those given the samples on arrival in the laboratory and are without significance as to grading.

TABLE 3.—Percentages of light of different wave lengths passing through the honeys derived from white and alsike clovers

Grade and honey No.	Blue light (480 mμ)	Yellow-green light (580 mμ)	Red light (680 mμ)	$\frac{R^*}{B}$	Grade and honey No.	Blue light (480 mμ)	Yellow-green light (580 mμ)	Red light (680 mμ)	$\frac{R^*}{B}$
Water White:	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>		Light Amber:	<i>Per ct.</i>	<i>Per ct.</i>	<i>Per ct.</i>	
93.....	61.0	77.0	75.0	1.23	201.....	54.0	83.0	90.5	1.68
Extra White:					56.....	48.0	70.0	75.0	1.56
307.....	54.0	69.0	74.0	1.37	265.....	48.0	71.0	80.0	1.67
90.....	75.0	90.0	95.0	1.27	274.....	33.0	54.0	65.0	1.97
191.....	70.0	86.0	93.0	1.33	273.....	25.0	45.0	58.0	2.32
135.....	58.5	73.0	79.0	1.35	112.....	36.5	56.5	64.5	1.77
White:					302.....	41.5	69.0	80.0	1.93
193.....	61.5	79.0	89.0	1.45	177.....	47.5	78.5	83.0	1.75
219.....	59.0	77.0	80.0	1.36	31.....	37.5	64.0	68.5	1.85
256.....	58.0	74.0	79.0	1.36	154.....	30.5	51.0	58.0	1.90
95.....	45.0	59.5	63.0	1.40	23.....	44.0	68.0	74.0	1.68
314.....	50.0	73.0	77.0	1.54	60.....	41.0	70.0	77.5	1.89
200.....	56.0	77.5	84.0	1.50	271.....	32.5	62.5	71.0	2.18
272.....	50.0	73.0	80.0	1.60	Extra Amber:				
152.....	49.5	70.0	75.0	1.52	45.....	31.0	61.0	67.0	2.16
4.....	58.0	77.0	80.5	1.39	68.....	25.0	61.0	74.0	2.96
27.....	55.5	76.0	80.0	1.44	96.....	25.0	52.0	63.5	2.54
61.....	56.0	76.0	80.0	1.43	216.....	19.5	53.0	61.0	3.13
153.....	52.5	74.0	81.0	1.54	192.....	22.0	59.0	73.0	3.32
129.....	52.0	73.5	80.0	1.54	121.....	21.0	51.5	62.0	2.95
149.....	48.5	64.0	73.0	1.51	184.....	13.0	42.0	52.0	4.00
62.....	36.0	63.0	71.0	1.97	38.....	5.5	17.5	24.0	4.36
Extra Light Amber:					30.....	1.0	36.5	66.0	66.00
236.....	56.0	84.0	93.0	1.66	Maximum.....	75.0	90.0	95.0	1.27
91.....	56.0	80.0	89.0	1.59	Minimum.....	1.0	17.5	24.0	24.00
79.....	56.5	78.0	84.5	1.50	Average.....	44.0	67.5	75.0	1.70
108.....	51.0	74.0	77.0	1.51					
110.....	54.0	81.5	84.0	1.56					
200.....	54.0	84.5	88.0	1.63					

* Ratio of transmission of red light to transmission of blue.

It is evident from Table 3, which shows but a small portion of the original tables, that the color gradations of these honeys, as determined by experienced handlers of honey, fail to correspond with the gradations in light transmission for any one of the three wave lengths measured. So great is the variation in light transmission in honeys of practically the same color that it is necessary to find some other way to indicate differences in color.

It may be assumed that the grading of the 290 samples meets the demands of the American market. If this assumption is correct, the light transmissions for the various primary and secondary grades in these samples may be accepted as characteristic of honeys of the respective grades. Table 4 gives the data in condensed form for all of the 290 samples.

TABLE 4.—Condensed data on light transmissions of the selected wave lengths for 290 honeys

Grade	Samples	Blue light transmitted			Yellow-green light transmitted			Red light transmitted			$\frac{R^*}{B}$ of individual samples		
		Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average	Maximum	Minimum	Average
	Number	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent	Per cent
Water White.....	24	80	53.0	66.2	92.5	67.5	77.2	96.0	73.5	82.5	1.43	1.15	1.25
Extra White.....	22	75	47.0	60.7	91	56.5	75.2	97.0	58.0	80.2	1.45	1.23	1.32
White.....	49	63	24.5	51.4	86.5	35.5	70.1	90.5	39.0	75.9	1.97	1.31	1.47
Extra Light Amber.....	13	56.5	40.0	50.4	84.5	62.5	74.4	93.0	70.0	80.9	1.85	1.48	1.61
Light Amber.....	68	54	23.5	39.1	86	41.0	65.6	90.5	50.5	73.9	2.43	1.56	1.90
Amber.....	64	41	4.0	21.3	82	17.5	55.4	92.0	24.0	66.8	14.75	3.56	3.62
Dark.....	30	22	0	3.25	88	5	23.1	78.0	12.0	42.1	(*)	2.45	-----

* Ratio of transmission of red light to transmission of blue.

* Infinity.

THE POSSIBLE EFFECT OF RELATIVE SENSITIVITY OF THE EYE TO THE PROBLEM

The human eye does not perceive lights of all wave lengths equally. Table 5, which is taken from the work of Coblentz and Emerson,⁹ shows the relative sensitivity of the eye to various colors. It is based on observations with 130 individuals made by detecting the disappearance of flicker when two lights of different colors and intensities were alternated rapidly.

TABLE 5.—Relative sensitivity of the eye to lights of various colors *

Wave length (m μ)	Visibility	Wave length (m μ)	Visibility	Wave length (m μ)	Visibility	Wave length (m μ)	Visibility
440.....	0.033	520.....	0.710	600.....	0.687	660.....	0.0645
460.....	.056	540.....	.954	620.....	.427	680.....	.0178
480.....	.125	560.....	.998	640.....	.194	700.....	.0040
500.....	.316	580.....	.898				

* After Coblentz and Emerson. Op. cit., Table 5, p. 219.

It is evident from Table 5 that the human eye perceives light most acutely in the region of 560 m μ (yellow-green) and much less acutely at both the violet (440 m μ) and red (700 m μ) ends of the visible spectrum.

It might be suspected that the relative sensitivity of the eye to lights of various colors has some effect on the color impressions arising from the passage of light through honeys. This seems not to be the case, however, for the relative sensitivity is the same for the light either before or after it passes through the honey. No results of value have been obtained from attempts to calculate the light transmission of the various honeys in relation to the relative sensitivity of the eye to colors, since nothing approaching regularity has been found in such computations.

THE RATIO OF TRANSMISSIONS AS A BASIS FOR GRADING

As previously stated, differential absorption of the white light gives the impression of colors that we get from looking at honeys. If the light which passes through honey has exactly the same relative proportions of the various wave-length components as are found in the original white light which strikes the honey, our impression would be that the honey is white. If some light is absorbed more than others, we get different color impressions, depending on which wave lengths of light pass without absorption.

Before discussing this subject in detail, reference may be made to the "color" often seen in honey from fireweed or willow herb (*Epilobium angustifolium*). This honey would usually be graded as Water White or Extra White, fireweed honey being one of the lightest to be found anywhere. A pure fireweed honey is so nearly devoid of amber

⁹ COBLENTZ, W. W., and EMERSON, W. B. RELATIVE SENSIBILITY OF THE AVERAGE EYE TO LIGHT OF DIFFERENT COLORS AND SOME PRACTICAL APPLICATIONS TO RADIATION PROBLEMS. U. S. Dept. Com., Bur. Standards Bul. 14: 167-236, illus., 1918.

color that it often has a slightly gray appearance. This appearance of grayness arises from the fact that all wave lengths of light are absorbed in almost the same proportions, and the light which passes through a stratum of this honey is therefore practically white light. There is, however, some absorption of lights of all wave lengths, so that the amount of light which passes through the honey is less than that which comes around a jar. This reduction in the light as it passes through the honey gives merely an impression of slight grayness, without any noticeable color. Even for fireweed honey there is always slightly greater absorption of blue than of red light, as indicated in Table 2.

It is, then, possible for a honey to give the impression of being white or slightly gray even though a considerable proportion of the light which strikes it is absorbed. Although entirely equal absorption of all wave lengths has not been found for any honey, the human eye gives a color value, however slight, to all honeys. Usually this color is described as some shade of amber, the implication being that the wave lengths of light toward the red end of the visible spectrum pass through the honey more readily than do those of the violet end. An examination of the data for the clover honeys (Table 3) shows that one honey allowed 95 per cent of the red light ($680\text{ m}\mu$) to pass through a stratum of 1 cm, whereas the greatest transmission of blue light ($480\text{ m}\mu$) was 75 per cent. The averages for the clover honeys were as follows: Blue ($480\text{ m}\mu$), 44 per cent; yellow-green ($580\text{ m}\mu$), 67.5 per cent; red ($680\text{ m}\mu$), 75 per cent. These figures support the previous statement that honeys permit more red light to be transmitted than any other, the violet end of the visible spectrum being most heavily absorbed.

It is possible for two honeys to have the same ratio of light transmission in the several wave lengths and still to absorb quite different percentages of the light of each of the three wave lengths. In such an event the honeys would have the same color, yet one would actually transmit less light than the other. As one goes toward the darker grades of honey, one finds that more and more light is absorbed but that there is a most rapid reduction in the transmission of light toward the violet end of the spectrum. It therefore seems at least theoretically possible to use as a factor in establishing the color grades of honey the ratio obtained by dividing the percentage transmission of red light by the percentage transmission of blue light. This ratio would be a constantly increasing number from the lightest to the darkest honeys.

The clover honeys have been examined on this basis. The lowest ratio found is 1.23 for the lightest honey, and the highest ratio is 66 for the darkest. (Table 3.) There is a steady increase in the ratio from the lightest to the darkest clover honey, but the rate of increase is not entirely uniform because of the smallness of the series. There are also certain variations which can not readily be explained on the basis of measurements with the spectrophotometer alone.

Since the actual figures representing the percentages of light of various wave lengths fail to give any satisfactory means of delimiting the color grades, and since the ratio of red to blue gives a more satis-

factory way of indicating these limits, it seems possible that the definitions of the honey color grades might be based chiefly on this ratio. The figures published by Sechrist and Samson ¹⁰ seem to be of some service, however, for while they can not be accepted as the lower limits of light transmissions for the several grades, they may still be useful as indicators of the percentage of light absorption to be expected toward the lower limits of the grades. It therefore seems wise not to abandon these figures entirely but to use them as examples. They will serve as a further check, in the event that later attempts are made to devise practical grading instruments for the use of beekeepers and honey dealers.

It may be admitted that no optical physicist would probably ever think of using such a ratio of light transmissions as is here suggested. However, since the ratio seems to serve as a useful method for defining the honey color grades, being far superior to any other attempt made to define them, there seems to be no valid reason for refusing to accept it merely because the method is unusual.

The reduction in light transmission is seen to be regularly such that the red exceeds the yellow-green and the yellow-green the blue. On the basis of the suggestion made earlier, it might then be possible to use the factor $\frac{R}{B}$, $\frac{R}{Y}$, or $\frac{Y}{B}$. These factors appear to be equally valid, but of course the one should be chosen that gives the most consistent and definite results. As might be expected, the extremes best meet this requirement.

It would also be possible to get still larger numbers for the ratio if wave lengths other than 480 $m\mu$ and 680 $m\mu$ were chosen. For example, if 440 $m\mu$ and 700 $m\mu$ were used, the ratio would be larger, but in that event the wave lengths measured would approach the limits of human vision, which would make the measurements less definite. Since the selection of any two measurements is wholly arbitrary, it is necessary only to get data on wave lengths far enough apart to show significant ratios.

By computing the three factors mentioned for all the honeys in averages for groups of tens, all light transmissions for each group being averaged, the usefulness of these three factors has been tested.

For $\frac{Y}{B}$ there are five instances in which a group of 10 honeys give a ratio on this basis which is lower than that of the group of 15 honeys immediately adjacent on the light side. For $\frac{R}{Y}$, there are 10 such exceptions. For $\frac{R}{B}$ there are found only 3, perhaps as good a showing as can be found for any grading scheme for honeys. These ratios are given in Table 6.

¹ SECHRIST, E. L., and SAMSON, H. W. Op. cit.

TABLE 6.—Average percentage light transmission for the lightest 260 honeys in groups of 10 each, with computed ratios as indicated

[The 30 darkest honeys are omitted]

Group No.	Percentage transmission of—			$\frac{R}{B}$	$\frac{R}{Y}$	$\frac{Y}{B}$
	Blue light	Yellow-green light	Red light			
1	70.3	80.1	86.0	1.23		1.14
2	62.7	74.2	78.9	1.26	^a 1.06	1.19
3	63.4	76.8	82.2	1.30	1.07	1.21
4	58.1	71.7	76.7	1.32	1.07	1.24
5	62.4	79.2	84.1	1.35	^a 1.00	1.27
6	58.7	75.1	81.5	1.39	1.10	1.29
7	50.8	69.0	74.2	1.46	^a 1.08	1.36
8	54.4	75.1	80.9	1.49	^a 1.08	1.38
9	45.5	64.7	70.5	1.55	^a 1.09	1.42
10	42.7	64.6	73.0	1.72	1.13	1.52
11	51.2	75.6	80.6	^a 1.58	^a 1.07	^a 1.48
12	43.8	66.6	75.0	1.73	1.13	1.53
13	40.6	66.3	75.0	1.88	1.14	1.64
14	41.4	67.2	74.8	^a 1.81	^a 1.12	^a 1.62
15	38.4	67.1	74.1	1.94	^a 1.11	1.75
16	37.7	64.2	74.2	1.99	1.16	^a 1.72
17	34.0	62.0	69.9	2.56	^a 1.13	1.83
18	32.6	63.0	71.7	2.20	^a 1.14	1.94
19	29.3	61.5	70.1	2.44	^a 1.15	2.13
20	25.0	58.0	68.5	2.84	1.19	2.39
21	24.8	55.6	65.6	^a 2.67	^a 1.18	^a 2.27
22	22.6	56.0	71.6	3.33	1.28	2.74
23	21.7	55.7	69.2	3.48	^a 1.25	^a 2.66
24	17.4	54.6	66.6	4.09	^a 1.23	3.35
25	14.5	47.0	60.5	4.34	1.30	3.35
26	11.4	47.0	60.4	6.32	^a 1.29	4.85

^a These ratios show irregularities in the progression from the lighter to the darker honeys.

If this proposed grading plan is subjected to still another test an examination of the ratios $\frac{R}{B}$ for the individual honeys of the entire series only six cases will be found of honeys which have a ratio higher than the average ratio of the next lower grade. These six exceptions should be contrasted with the 263 exceptions found on examination of the definitions of grades formerly published. Three of these exceptions occur in the Water White grade and three in the White grade. None appear in the darker grades, and in both instances the exceptions occur because of the interpolation of secondary (extra) grades.

CONCLUSIONS

As a result of a study of the transmission of light of three wave lengths through 290 honeys which had been arranged in series and marked as to their proper market grade by expert honey graders, the definitions for honey color grades shown in Table 7 are proposed.

TABLE 7.—*Proposed definitions of honey color grades based on transmission of blue, yellow-green, and red light and on the ratios between the transmissions of the red and blue lights*

Grade	Approximations of light transmission for the lower limits of the grades			Ratio of red light transmission to blue $\left(\frac{R}{B}\right)$	
	Blue light (480 m μ)	Yellow-green light (580 m μ)	Red light (680 m μ)	Maximum	Average
	<i>Per cent</i>	<i>Per cent</i>	<i>Per cent</i>		
Water White.....	64.0	77.0	81.5	1.43	1.25
Extra White.....	60.0	76.0	80.5	1.45	1.32
White.....	50.0	71.0	78.0	1.61	1.47
Extra Light Amber.....	48.0	70.0	77.0	1.67	1.61
Light Amber.....	34.5	63.0	73.0	2.43	1.90
Extra Amber.....	26.0	60.5	70.5	^a 4.20	(^a)
Amber.....	15.0	49.0	63.0	^a 8.00	3.62
Dark Amber.....	6.0	43.0	59.0	^a 9.83	(^a)
Dark ^b	(b)	(b)	(b)	(c)	?

^a Averages are not given for the grades Extra Amber and Dark Amber, since no definite division points for these grades were designated in the 290 honeys studied. The maximum ratios are based on estimates of the probable maximum if the secondary grades are to include roughly 30 per cent of the primary grades.

^b All honeys darker than Amber (or Dark Amber).

^c Infinity.

The figures in Table 7 for maximum ratio of red light transmission to blue light transmission differ from those given in Table 4 for the same grades in three instances. These changes are proposed because the extreme figures for ratios in Table 4 were those of three honeys toward the lower end of the respective grades (White, Extra Light Amber, and Amber) which may as well be allowed to fall into darker grades. In no case did these exceptional honeys lie farther than two or three from the darker limits of the grades. These changes are proposed also because the ratios of the particular honeys which gave abnormally high ratios are considerably higher than those of honeys immediately adjacent to them in the series.

If color-grading rules based on these figures are adopted, it is believed that it will be possible for any properly equipped manufacturing concern to make a honey color grader that will meet the demands of the definitions. Moreover, the color grades will be accurately defined, which so far has not been the case.

INDEX

	Page		Page
Abortion—		Bark beetles, studies.....	437-444
disease of cows, iodine treatment, experi- ments.....	111, 114-117, 126-127	BEADLES, JESSIE R.; MITCHELL, H. H.; HAMILTON, T. S.; McCURE, F. J.; HAINER, W. T.; and MORRIS, H. P.: The Effect of the Amount of Feed Consumed by Cattle on the Utilization of Its Energy Content.....	163-191
infectious, immunizing agents, <i>Brucella</i> <i>abortus</i> , efficacy of different strains against, W. E. Cotton.....	705-724	Bean—	
Agglutinin, content of blood, effects of vac- cination and exposure.....	711-715, 720-723	plants, characters, description.....	7-16
Agropyron, four species, identification key.....	371	pods, characters, description.....	16-24
<i>Aleurites fordii</i> . See Tung-oil tree.		seedlings, characters, description.....	6-7
ALLEN, RUTH F.: A Cytological Study of Heterothallism in <i>Puccinia coronata</i>	513-541	Beans—	
Amylase, in Colorado potato beetle, study.....	473-474, 480, 481	field—	
Anthraxnose—		(<i>Phaseolus vulgaris</i>), varieties, classifica- tion, F. H. Steimmetz and A. C. Army.....	1-30
northwestern, of apple, pathogene, physi- ological studies.....	65-76	varieties, classification and factors affect- ing.....	1-49
rose, caused by <i>Sphaeceloma</i> , Anna E. Jenkins.....	321-337	varieties grown in Minnesota, descrip- tion.....	29-49
<i>Aphelechioides</i> —		seed size, effect of environment, experi- ment.....	3-6
<i>acroposthion</i> , n. sp., taxonomy.....	443-444	BECHDEL, S. I.; FORBES, E. B.; KARNIS, GEO. M.; WILLIAMS, P. S.; KEITH, T. B.; CALLENBACH, E. W.; and MURPHY, R. R.: The Value of Iodine for Livestock in Central Pennsylvania.....	111-128
<i>conurus</i> , n. sp., taxonomy.....	441-442	Bee colonies, inoculation with <i>Bacillus lar-</i> <i>vae</i> , methods and observations.....	259-266, 282-283
spp., ecological groups.....	438-439, 444	Beekeeping industry, effect of spread of American foulbrood on.....	257-283
status and generic characters.....	441-442, 444	BENNETT, E.; ARCHBOLD, J. G.; and NEL- SON, T. H.: A Three-Year Study of the Chemical Composition of Grass from Plots Fertilized and Grazed Intensively.....	627-640
Apple—		Blood of cows, agglutinin content, effects of vaccination and exposure.....	711-715, 720-723
anthraxnose, northwestern, pathogene, physiological studies.....	65-76	Bollworms, pink, detection in cottonseeds by X ray. F. A. Fenton and Willis W. Walte.....	347-348
perennial canker pathogene, physiological studies.....	65-76	<i>Brassica oleracea</i> . See Cabbage.	
rots, produced by <i>Gloeosporium petenans</i> and <i>Neofabraea malicorticis</i> , study.....	72-73	Bread, of hard red spring wheat flour, loaf volume and weight, protein content, and water absorption, studies.....	209-231
ARCHBOLD, J. G.; NELSON, T. H.; and BENNETT, E.: A Three-Year Study of the Chemical Composition of Grass from Plots Fertilized and Grazed Intensively.....	627-640	Breeding, wheat, for resistance to bunt, ex- periments.....	501-505
ARNY, A. C., and STEINMETZ, F. H.: A Classification of the Varieties of Field Beans, <i>Phaseolus vulgaris</i>	1-50	BRIGGS, FRED N.: Inheritance of Resist- ance to Bunt, <i>Tilletia tritici</i> , in Hybrids of White Federation and Odessa Wheat.....	501-505
Arsenicals, effect on activity of digestive en- zymes of Colorado potato beetle.....	479-481	Br. megrass, growth, comparison with crested wheatgrass.....	373-377, 378-382, 383
Asparagus—		BROOKS, CHARLES, and MILLER, ERSTON V.: Effect of Carbon Dioxide Content of Storage Atmosphere on Carbohydrate Transformation in Certain Fruits and Vegetables.....	449-459
cutting season, length, effect on yields.....	105-108, 109	<i>Brucella abortus</i> —	
plant, sex, effect on yield.....	102-105, 108-109	infection, dairy cows, treatment with iodine, experiments.....	111, 114-117, 126-127
size of crown, relation to yield.....	102-105, 108-109	strains, efficacy as immunizing agents against infectious abortion. W. E. Cot- ton.....	705-724
yields, effect of size of crown and length of cutting season. E. S. Haber.....	101-109	BULL, HENRY, and SCHUMACHER, FRANCIS X.: Determination of the Errors of Esti- mate of a Forest Survey, with Special Reference to the Bottom-Land Hardwood Forest Region.....	741-756
Atmosphere, storage, carbon dioxide con- tent, effect on carbohydrate transforma- tion in certain fruits and vegetables. Er- ston V. Miller and Charles Brooks.....	449-459	Bull, dairy—	
Azotobacter—		ante-mortem—	
production of nitrogen changes in certain nitrogenous compounds and the nitro- gen fixed in the presence of these com- pounds. L. G. Thompson, Jr.....	149-161	external measurements, comparison with cow, data.....	642-650
spp., production of nitrogen changes, ex- periments and discussion.....	140-160	post-mortem and skeletal structure data, interrelations.....	669-670, 674
<i>Bacillus</i> —			
<i>amylolovor</i> —			
cause of disease on fruit, studies.....	59-63		
migration in the tissues of the quince. Herbert A. Wahl.....	59-64		
larvae, spores—			
in commercial honey, investigation.....	258, 278-282, 283		
numbers necessary to produce disease in bee colonies, experiments.....	258-264, 282-283		
production of vegetative growth in cul- tures, experiments.....	267-278		
<i>Bacterium aleuritidis</i> , n. sp.—			
infection of tung-oil tree.....	339-346		
morphology, characters, and technical description.....	344-346		

	Page		Page
Bull, dairy—Continued.		Cotton root-rot—	
conformation, anatomy, and skeletal structure, comparison with dairy cow.		fungus, distribution in soil and in plant tissues, relation to control by disinfectants.	
W. W. Swett, R. R. Graves, and Fred W. Miller.	641-674	C. J. King and Claude Hope.	725-740
post-mortem measurements, study.	650-653	spread prevention by mapping infested area.	726-728
skeletal measurements, data.	653-669, 672-673	Cottonseeds, pink bollworms in, detection by X ray.	
Bunt, <i>Tilletia tritici</i> , resistance of White Federation and Odessa wheat hybrids to, inheritance.	501-505	F. A. Fenton and Willis W. Waite.	347-348
Butterfat, quantity obtained from ante-mortem and post-mortem milkings, comparison.	405-406, 411-418	Cow—	
Cabbage—		dairy—	
bottom rot, description.	461-462	ante-mortem external measurements, comparison with bull, data.	642-650
inoculation with <i>Corthium vagum</i> .	464-466	ante-mortem, post-mortem, and skeletal structure data, interrelations.	669-670, 674
Rhizoctonia—		conformation, anatomy, and skeletal structure, comparison with dairy bull.	
bottom rot and head rot.	F. L. Wellman.	W. W. Swett, R. R. Graves, and Fred W. Miller.	641-674
head rot, description and comparison with other head rots.	461-469	post-mortem measurements, study.	650-653
Calcium, diet balances for heifers, digestibility determinations.	558, 560-562	skeletal measurements, data.	653-669, 672-673
CALLENBACH, E. W.; FORRES, E. B.; KARN, GEO. M.; BECHDEL, S. I.; WILLIAMS, P. S.; KEITH, T. B.; and MURPHY, R. R.: The Value of Iodine for Livestock in Central Pennsylvania.	111-128	udders—	
Calves, feeding with iodine, experiments.	112-113, 121, 127	amputated, composition of milk obtained from.	
Canker, perennial, of apple, pathogene, physiological studies.	65-76	W. W. Swett, Fred W. Miller, and R. R. Graves.	401-419
Carbohydrate transformation, fruits and vegetables, effect of carbon dioxide content of storage atmosphere on.	Erston V. Miller and Charles Brooks.	amputated, quantity of milk obtained from.	
Carbon dioxide content of storage atmosphere, effect on carbohydrate transformation in certain fruits and vegetables.	149-459	W. W. Swett, Fred W. Miller, and R. R. Graves.	385-400
Erston V. Miller and Charles Brooks.	149-459	characteristics, relation to milk production, study.	577-607
Caseln, effect on serum layer of cream.	485, 498	quality, size, capacity, gross anatomy, and histology, relation to milk production.	
Cattle—		W. W. Swett, Fred W. Miller, R. R. Graves, and G. T. Creech.	577-607
dairy—		Cows—	
conformation, anatomy, and skeletal structure, comparisons.	641-674	dairy—	
feeding on artificially dried herbage, experiments.	557-562	milk secretion studies.	385-399, 401-418
feed—		rations, influence on chemical and physical properties of milk fat.	51-58
consumption, effect of amount on utilization of energy content.	H. H. Mitchell, T. S. Hamilton, F. J. McClure, W. T. Haines, Jessie R. Beadles, and H. P. Morris.	feeding with	
energy content, utilization, relation to amount of feed consumed.	163-191	alfalfa hay, experiments.	507-511
H. H. Mitchell, T. S. Hamilton, F. J. McClure, W. T. Haines, Jessie R. Beadles, and H. P. Morris.	163-191	iodine for abortion disease, experiments.	111, 114-117, 126-127
nutrition, studies.	163-190	immunization against <i>Brucella abortus</i> , experiments.	703-724
Cherries, reaction to carbon dioxide gas, study.	450-452, 455-458	milk production	
Chickens—		ante-mortem and post-mortem milkings, comparison.	398-399
feeding with iodine, experiments.	112-113, 124-126, 128	relation to quality, size, capacity, gross anatomy, and histology of udders.	
lead arsenate poisoning in.	E. F. Thomas and A. L. Shealy.	W. W. Swett, Fred W. Miller, R. R. Graves, and G. T. Creech.	577-607
Chloroplasts, in potato—		vaccination, effects on blood stream, experiments.	711-724
division, description.	430-433	Cream—	
origin, development, and increase.	Winnom E. Stone.	bottled, separation of serum from, study of factors.	G. Malcolm Trout and J. C. McCan.
Cold storage—		heat treatment, effect on serum layer.	489, 499
cream, effect on formation of serum layer.	493	serum layer, formation, cause and prevention, study.	483-500
peas, effects on keeping quality and flavor, experiments.	361-370	viscosification for reduction of serum layer, experiments.	495-496, 499
Color grading, honey, importance.	757-758	CREECH, G. T.; SWETT, W. W.; MILLER, FRED W.; and GRAVES, R. R.: Quality, Size, Capacity, Gross Anatomy, and Histology of Cow Udders in Relation to Milk Production.	577-607
Corn, sweet—		Crown rust. See <i>Puccinia coronata</i> .	
mean increase in yields per acre in fertilized plots, experiments, analysis.	676-704	Dairy cows. See Cows, dairy.	
reaction to carbon dioxide gas, study.	450-452, 453-454, 457-458	DANIEL, ESTHER PETERSON, and MUNSSELL, HAZEL E.: The Vitamin A, B, C, and G Content of Concord Grapes.	445-448
<i>Corthium vagum</i> , cause of Rhizoctonia rots of cabbage.	465-469	DEMARKEE, J. R., and McCULLOCH, LUCIA: A Bacterial Disease of the Turp-Oil Tree.	339-346
COTTER, RALPH U., and LEVINE, MOSES N.: Physiologic Specialization in <i>Puccinia graminis secalis</i> .	297-315	<i>Dendroctonus monticolae</i> , mountain pine beetle, nematode parasites and associates.	G. Steiner.
COTTON, W. E.: Efficacy of Different Strains of <i>Brucella abortus</i> as Immunizing Agents against Infectious Abortion.	705-724	DICKSON, JAMES G.; PUGH, GRACE WINELAND; and JOHANN, HELEN: Relation of the Semipermeable Membranes of the Wheat Kernel to Infection by <i>Gibberella saubinetii</i> .	609-626
		Digestion, steers, experiments.	166-167, 172-173
		Digestive enzymes of Colorado potato beetle and influence of arsenicals on their activity.	David E. Fink.
		<i>Diplogaster occidentalis</i> , n. sp., taxonomy.	439-441, 444

	Page		Page
Disaccharides, hydrolysis by enzymes of Colorado potato beetle	474-476	Fruit moth, oriental, parasite of, <i>Macrocetrus ancyrothrus</i> Roh. (G. J. Haussler)	79-100
Driers, for plants, kinds and advantages ..	507	Fruits, carbohydrate transformation, effect of carbon dioxide content of storage atmosphere on .. Erston V. Millet and Charles Brooks	449-459
Drying, artificial, of alfalfa hay, effect on availability of nutrients .. E. B. Hart, O. L. Kline, and G. C. Humphrey	507-511	Fungi, blue stain, prevention, cause of chemical stains of wood	233-237
DULEY, F. L., and HAYS, O. E.: The Effect of the Degree of Slope on Run-Off and Soil Erosion ..	349-360	Fusarium stains of lumber and logs, study ..	234-237
Energy content of feed consumed by cattle, utilization, relation to amount consumed .. H. H. Mitchell, T. S. Hamilton, F. J. McClure, W. T. Haines, Jessie R. Beadles, and H. P. Morris ..	163-191	GAINES, P. L., and SEWELL, M. C.: The Role of Nitrogen in the Production of Spots in Wheat Fields	129-148
Enzymes—		GARRETT, O. E., and OVERMAN, O. R.: The Influence of Certain Balanced Rations on the Chemical and Physical Properties of Milk Fat	51-58
digestive, of Colorado potato beetle, influence of arsenicals on their activity .. David E. Fink	471-482	Gelatin—	
proteolytic, of Colorado potato beetle, study ..	472-473, 476-479, 480, 481	digestion by enzymes of Colorado potato beetle	477-479
Erepsin, in Colorado potato beetle ..	479	effect on serum layer in cream, experiments ..	485, 497-498, 500
Erosion, soil, and run-off, effect of degree of slope on .. F. L. Duley and O. E. Hays ..	349-350	<i>Gibberella subnigritica</i> —	
Eye, sensitivity to lights of various colors ..	736	distribution within wheat kernels ..	622-621, 625
Fasting steers, heat production, experiments ..	180-181, 183-186, 189	infection, relation to semipermeable membranes of the wheat kernel, Grace Wineland Pugh, Helen Johann, and James G. Dickson ..	609-626
Fat, milk, chemical and physical properties, influence of certain balanced rations .. O. R. Overman and O. F. Garrett ..	51-58	Gipsy moth, Tachinid parasite, <i>Stenomacropis Meigen</i> , R. T. Webber ..	193-208
Fatty acids, formation in liver ..	230, 251	<i>Gliocladium</i> sp., staining of wool, study ..	235, 237
Feed consumed by cattle, amount, effect on utilization of its energy content .. H. H. Mitchell, T. S. Hamilton, F. J. McClure, W. T. Haines, Jessie R. Beadles, and H. P. Morris ..	163-191	<i>Gloeosporium perennans</i> and <i>Neofabraa malitioris</i> , physiological studies, Erston V. Millet ..	65-77
Feeding—		Grain mixtures, effect on milk fat production of dairy cows, data ..	51-57
cows, on alfalfa hay, experiments ..	507-511	Grapes, Concord, Vitamin A, B, C, and G content, Esther Peterson Daniel and Hazel E. Munsell ..	445-448
dairy cattle, with artificially dried pasture herbage, experiments ..	557-562	<i>Grapholitha modesta</i> , development during parasitism by <i>Macrocetrus ancyrothrus</i> ..	87-88
dairy cows, for milk fat production, experiments and results ..	51-57	Grass—	
FENTON, F. A., and WAITE, WILLIS W.: Detecting Pink Bollworms in Cotton-seeds by the X Ray ..	347-348	chemical composition, three-year study ..	627-640
Fertilizer ratios, optimum, efficiency factors and their use in determining .. W. A. Huelshen ..	67-70	from plots fertilized and grazed intensively, chemical composition, a three-year study .. J. G. Archibald, P. R. Nelson, and E. Bennett ..	627-640
Fertilizers, effect on composition of pasture grass ..	610-612, 636-639	GRAVES, R. R., SWETT, W. W., and MILLER, FRED W.: Comparison of Conformation, Anatomy, and Skeletal Structure of the Cow and Bull of a Dairy Breed ..	641-674
Field beans .. See Beans, field.		Composition of Milk Obtained from Amputated Cow Udders ..	401-419
FINK, DAVID E.: The Digestive Enzymes of the Colorado Potato Beetle and the Influence of Arsenicals on Their Activity ..	471-482	Quantity of Milk Obtained from Amputated Cow Udders ..	385-400
Fire-blight organism, cause of disease in quince, studies ..	59-63	MILLER, FRED W., and CREECH, G. T.: Quality, Size, Capacity, Gross Anatomy, and Histology of Cow Udders in Relation to Milk Production ..	577-607
Flax—		Grazing, effect on composition of pasture grass, study ..	630-632, 639, 640
boll, size, increase on soils of different productivity, study ..	243-245, 248-249, 253, 254	GREEN, E. L., and KERTESZ, Z. L.: Deterioration in Shelled Green Peas Held a Few Days in Storage Prior to Canning ..	361-370
planting, delayed, influence on yield and composition of seed ..	239, 250-253, 254	Gum, red, lumber of, inoculation treatment for blue stain, study ..	233-237
development rate, study ..	239, 243-249, 253, 254		
iodine absorption number, determination ..	243, 249, 254	HABER, E. S.: Effect of Size Crown and Length of Cutting Season on Yields of Asparagus ..	101-109
oil in, quantity and quality, relation to agronomic practice .. I. J. Johnson ..	239-255	HAUSSLER, G. J.: <i>Macrocetrus ancyrothrus</i> Roh., an Important Parasite of the Oriental Fruit Moth ..	79-100
Flour—		HAINES, W. T., MITCHELL, H. H., HAMILTON, T. S., MCCLURE, F. J., BEADLES, JESSIE R., and MORRIS, H. P.: The Effect of the Amount of Feed Consumed by Cattle on the Utilization of Its Energy Content ..	163-191
baking tests ..	209-230	HAMILTON, T. S., MITCHELL, H. H., MCCLURE, F. J., HAINES, W. T., BEADLES, JESSIE R., and MORRIS, H. P.: The Effect of the Amount of Feed Consumed by Cattle on the Utilization of Its Energy Content ..	163-191
protein content, correlation studies ..	212-214, 215, 223, 227, 228, 229	HANSON, HERBERT C., and LOVE, L. DUDLEY: Life History and Habits of Crested Wheatgrass ..	371-383
FORBES, E. H.; KARNS, GEO. M.; BRIDDEL, S. L.; WILLIAMS, P. S.; KEITH, T. B.; CALLENBACH, E. W.; and MURPHY, R. R.: The Value of Iodine for Livestock in Central Pennsylvania ..	111-128		
Forest—			
area, analysis, data ..	743-752		
survey, errors of estimate, determination, with special reference to the bottomland hardwood forest region .. Francis X. Schumacher and Henry Bull ..	741-756		
Formaldehyde, use for disinfection of soil infested with root-rot fungus ..	725, 728-730, 738-739		
Foulbrood, American, spread, relation to commercial honey to .. A. P. Sturtevant ..	257-285		
Freezing, potatoes, effect on sugar content ..	552, 553, 554-555		

	Page		Page
Hardwood—		Iodine—	
forest region, bottom-land, survey, errors of estimate, determination, Francis X. Schumacher and Henry Bull.....	741-756	absorption number in flaxseed, determination.....	243, 249, 254
lumber and logs, some minor stains, T. C. Scheffer and J. M. Lindgren.....	233-237	value for livestock in central Pennsylvania, E. B. Forbes, (Geo. M. Karns, S. I. Bechdel, P. S. Williams, T. B. Keith, E. W. Callenbach, and R. K. Murphy.....	111-128
HART, E. R.; KLINE, O. L.; and HUMPHREY, G. C.: The Effect of Artificial Drying on the Availability of the Nutrients of Alfalfa Hay.....	507-511	JENKINS, ANNA E.: Rose Anthracnose Caused by <i>Sphacelotheca</i>	321-337
Hay, alfalfa—		JOHANN, HELEN; PUGH, GRACE WINELAND; and DICKSON, JAMES G.: Relation of the Semipermeable Membranes of the Wheat Kernel to Infection by <i>Gibberella saubinetii</i>	609-626
digestibility, relation to method of drying, nutrients, availability, effect of artificial drying, E. B. Hart, O. L. Kline, and G. C. Humphrey.....	507-511	JOHNSON, I. J.: The Relation of Agronomic Practice to the Quantity and Quality of the Oil in Flaxseed.....	239-255
HAYS, O. E., and DULEY, F. L.: The Effect of the Degree of Slope on Run-off and Soil Erosion.....	349-360	Kafir, reed, inoculation with <i>Sphacelotheca</i> spp.....	288
Heat production—		KARNS, GEO. M.; FORBES, E. B.; BECHDEL, S. I.; WILLIAMS, P. S.; KEITH, T. B.; CALLENBACH, E. W.; and MURPHY, R. R.: The Value of Iodine for Livestock in Central Pennsylvania.....	111-128
in cattle, relation to type of feed consumed, studies and discussion.....	180-190	KEITH, T. B.; FORBES, E. B.; KARNS, GEO. M.; BECHDEL, S. I.; WILLIAMS, P. S.; CALLENBACH, E. W.; and MURPHY, R. R.: The Value of Iodine for Livestock in Central Pennsylvania.....	111-128
steers, during fasting, measurement.....	183-185, 189	KERNER, Z. I., and GREEN, E. L.: Detoxication in Shelled Green Peas Held a Few Days in Storage Prior to Canning.....	361-370
Heifers, dairy, on artificially dried pasture herbage, nitrogen, calcium, and phosphorus balance.....	557-562	KING, C. J., and HOPE, CLAUDE: Distribution of the Cotton Root-Rot Fungus in Soil and in Plant Tissues in Relation to Control by Disinfectants.....	725-740
Herbage, pasture, artificially dried, apparent digestibility, and nitrogen, calcium, and phosphorus balance of dairy heifers on, J. C. Knott and R. E. Hodgson.....	557-563	KLINE, O. L.; HART, E. B.; and HUMPHREY, G. C.: The Effect of Artificial Drying on the Availability of the Nutrients of Alfalfa Hay.....	507-511
Heterothallism—		KNOTT, J. C., and HODGSON, R. E.: Apparent Digestibility of, and Nitrogen, Calcium, and Phosphorus Balance of Dairy Heifers on, Artificially Dried Pasture Herbage.....	557-563
and hybridization in <i>Sphacelotheca sorghi</i> and <i>S. cruenta</i> , H. A. Rodenhiser.....	287-296	Lactase, in Colorado potato beetle, study.....	475,
crown rust of oats, greenhouse data.....	521-523	480, 481	
in <i>Puccinia coronata</i> , cytological study, Ruth F. Allen.....	513-541	Lactation, stage, variation, importance.....	591, 606
HODGSON, R. E., and KNOTT, J. C.: Apparent Digestibility of, and Nitrogen, Calcium, and Phosphorus Balance of Dairy Heifers on, Artificially Dried Pasture Herbage.....	557-563	Lambs, fattening, value of supplemental iodine, experiments.....	112-113, 122-124,
Honey—		127-128	
absorption and dispersal of light.....	762-763	Land, slope, effect of degree on run-off and soil erosion, F. L. Duley and O. E. Hays.....	349-360
color—		Lead—	
effect of light.....	762-763	arsenate—	
grades, definitions, E. F. Phillips.....	757-770	effect on activity of digestive enzymes of Colorado potato beetle.....	479-481
grading, importance.....	757-758	feeding to chickens, experiments and results.....	317-319
commercial—		poisoning in chickens, E. F. Thomas and A. L. Sheely.....	317-319
examination for spores of <i>Bacillus larvae</i>	278-282, 283	poisoning, symptoms, discussion.....	317-319
relation to spread of American foulbrood, A. P. Sturtevant.....	257-285	<i>Leptinotarsa decemlineata</i> , digestive enzymes, influence of arsenicals on their activity.....	471-482
HUTCHINSON, W. A.: Efficiency Factors and Their Use in Determining Optimum Fertilizer Ratios.....	675-704	LEVINE, MOSES N., and COTTER, RALPH U.: Physiologic Specialization in <i>Puccinia graminis secalis</i>	297-315
HOPE, CLAUDE, and KING, C. J.: Distribution of the Cotton Root-Rot Fungus in Soil and in Plant Tissues in Relation to Control by Disinfectants.....	725-740	Light—	
HUMPHREY, G. C.; HART, E. B.; and KLINE O. L.: The Effect of Artificial Drying on the Availability of the Nutrients of Alfalfa Hay.....	507-511	absorption and dispersal by honey.....	762-765
Hybridization and heterothallism in <i>Sphacelotheca sorghi</i> and <i>S. cruenta</i> , H. A. Rodenhiser.....	287-296	effect on—	
Hydrogen-ion—		greening of potato tubers.....	423-424
concentration and oxidation-reduction potentials of a soil, L. G. Willis.....	571-575	sensitivity of eye to colors.....	766
concentrations, effect on growth of <i>Gloeosporium perennans</i> and <i>Neofabraea malicortici</i>	67-68, 75-76	transmission, measurement for grading honey.....	763-769
Hyperparasites of <i>Sturmia inconspicua</i>	205, 208	Liming, soil, effect on oxidation reduction potential, data and discussion.....	571-574, 575
Inbreeding coefficients, calculation from livestock pedigrees, approximate method, empirical test, Jay L. Lush.....	565-569	LINDGREN, R. M., and SCHEFFER, T. C.: Some Minor Stains of Southern Pine and Hardwood Lumber and Logs.....	233-237
Inoculation—		Linseed meal, iodized, feeding to livestock, experiments.....	112-128
apples, with strains of <i>Gloeosporium perennans</i> and <i>Neofabraea malicortici</i> , study.....	73-75	Lipase, in Colorado potato beetle, study.....	476,
bees, with <i>Bacillus larvae</i> , experiments.....	259-266,	480, 481	
283-283			
quince, with <i>Bacillus amylovorus</i> , cytological effects.....	60-63		
reed kafir sorghum with <i>Sphacelotheca</i> spp. wood blocks, with fungi in study of stains.....	234-237		
Invertase, in Colorado potato beetle, study.....	475-476, 480, 481		

	Page		Page
Livestock—		MILLER, ERNSTON V.—	
breeding, experiments.....	565-569	Some Physiological Studies of <i>Glocosporium perennans</i> and <i>Neofabraea malicorticis</i>	65-77
iodine for, value in central Pennsylvania. E. B. Forbes, Geo. M. Karns, S. I. Bechdel, P. S. Williams, T. B. Keith, E. W. Callenbach, and R. R. Murphy, pedigrees, inbreeding and relationship coefficients, empirical test of the approximate method of calculating. Jay L. Lush.....	111-128	and BROOKS, CHARLES: Effect of Carbon Dioxide Content of Storage Atmosphere on Carbohydrate Transformation in Certain Fruits and Vegetables.....	449-459
relationship coefficients, calculation, tests.....	565-569	MILLER, FRED W.—	
Logs, southern pine and hardwood, some minor stains. T. C. Scheffer and R. M. Lindgren.....	233-237	GRAVES, R. R.; and SWETT, W. W.: Comparison of Conformation, Anatomy, and Skeletal Structure of the Cow and Bull of a Dairy Breed.....	641-674
LOVE, L. DUDLEY, and HANSON, HERBERT C.: Life History and Habits of Crested Wheatgrass.....	371-388	SWETT, W. W.; and GRAVES, R. R.—	
Lumber, southern pine and hardwood, some minor stains. T. C. Scheffer and R. M. Lindgren.....	233-237	Composition of Milk Obtained from Amputated Cow Udders.....	401-419
LUSH, JAY L.: An Empirical Test of the Approximate Method of Calculating Coefficients of Inbreeding and Relationship from Livestock Pedigrees.....	565-569	Quantity of Milk Obtained from Amputated Cow Udders.....	385-400
Macrocentrus—		SWETT, W. W.; GRAVES, R. R.; and CREECH, G. T.: Quality, Size, Capacity, Gross Anatomy, and Histology of Cow Udders in Relation to Milk Production.....	577-607
ancylinorus—		MICHAEL, H. H.; HAMILTON, T. S.; McCLURE, F. J.; HAINES, W. T.; BEADLES, JESSIE R.; and MORRIS, H. P.: The Effect of the Amount of Feed Consumed by Cattle on the Utilization of Its Energy Content.....	163-191
adult, description and biology.....	82-87	Mitosis, in potato chloroplasts, description. Moisture, effect on Rhizoctonia diseases of cabbage.....	430-433
life history and development.....	87-97, 99-100	MORRIS, H. P.; MITCHELL, H. H.; HAMILTON, T. S.; McCLURE, F. J.; HAINES, W. T.; and BEADLES, JESSIE R.: The Effect of the Amount of Feed Consumed by Cattle on the Utilization of Its Energy Content.....	467, 469
number of generations and hibernation.....	97-99, 100	MUNSELL, HAZEL E., and DANIEL, ERIK PETERSON: The Vitamin A, B, C, and G Content of Concord Grapes.....	445-448
Roh., an important parasite of the oriental fruit moth. G. J. Haussler.....	79-100	MURPHY, R. R.; FORBES, E. B.; KARNs, GEO. M.; BECHDEL, S. I.; WILLIAMS, P. S.; KEITH, T. B.; and CALLENBACH, E. W.: The Value of Iodine for Livestock in Central Pennsylvania.....	111 128
delicatus, similarly to <i>M. ancylinorus</i>	82-83	NELSON, P. R.; ARCHIBALD, J. G.; and BENNETT, E.: A Three-Year Study of the Chemical Composition of Grass from Plots Fertilized and Grazed Intensively.....	627-640
Maltaise, in Colorado potato beetle, study.....	475, 480, 481	<i>Neofabraea malicorticis</i> and <i>Glocosporium perennans</i> , physiological studies. Erston V. Miller.....	65-77
MANGELS, C. E., and WALDRON, L. R.: Correlational and Allied Studies of the Protein Content, Water Absorption, Leaf Volume, and Leaf Weight of Two Series of Hard Red Spring Wheats.....	209-231	Nitrogen—	
MCCAN, J. C., and TROUT, G. MALCOLM: A Study of Several Factors in the Separation of Serum from Bottled Cream.....	483-500	changes produced in certain nitrogenous compounds by Azotobacter and the nitrogen fixed in the presence of these compounds. L. G. Thompson, jr.....	149-161
McCLURE, F. J.; MITCHELL, H. H.; HAMILTON, T. S.; HAINES, W. T.; BEADLES, JESSIE R.; and MORRIS, H. P.: The Effect of the Amount of Feed Consumed by Cattle on the Utilization of Its Energy Content.....	163-191	diet balances for heifers, digestibility determination.....	558, 560-562
McCULLOCH, LUCIA, and DEMAREE, J. B.: A Bacterial Disease of the Tung-Oil Tree.....	339-348	effect on—	
Metabolism—		efficiency of phosphorus in corn production.....	681-684, 703-704
cows fed on alfalfa hay, experiments.....	510-511	efficiency of potash in corn production.....	687-689, 698-699
experiments with heifers.....	559-562	yield of corn, experiments.....	676-704
steer, at different levels of feeding, study of energy utilization.....	173-189	fixation, by Azotobacter, studies.....	149-161
Migration of <i>Bacillus amylovorus</i> in the tissue of the quince. Herbert A. Wahl.....	59-64	role in production of spots in wheat fields. P. L. Gainey and M. C. Sewell.....	129-148
Milk—		studies, in wheat fields, data for 1929, 1930, 1931.....	131-142
composition—		Nitrogenous compounds, nitrogen changes produced by Azotobacter and the nitrogen fixed in their presence. L. G. Thompson, jr.....	149-161
ante-mortem and post-mortem milkings, comparison.....	404-418	Oats, crown rust—	
obtained from amputated cow udders. W. W. Swett, Fred W. Miller, and R. R. Graves.....	401-419	heterothallism, studies.....	513-540
evaporated, effect on serum layer in cream, experiments.....	485, 498, 500	life cycle, data.....	515-521, 539-540
fat, chemical and physical properties, influence of certain balanced rations. O. R. Overman and O. F. Garrett.....	51-58	Oil—	
heat treatment, effect on formation of serum layer in cream.....	486-489, 499	content of flax, relation to productivity of soil.....	239, 243, 249-250, 253-254
powder, effect on serum layer in cream, experiments.....	485, 498, 500	in flaxseed, quantity and quality, relation to agronomic practice. I. J. Johnson.....	239-255
production.....		OVERMANN, O. R., and GARRETT, O. F.: The Influence of Certain Balanced Rations on the Chemical and Physical Properties of Milk Fat.....	51-58
effect of artificially dried hay, study.....	510-511		
relation to quality, size, capacity, gross anatomy, and histology of cow udders. W. W. Swett, Fred W. Miller, R. R. Graves, and G. T. Creech.....	577-607		
quantity obtained from amputated cow udders. W. W. Swett, Fred W. Miller, and R. R. Graves.....	385-400		
recovery from amputated cow udders.....	579-580, 583-584, 587-588		
secretion in cow udders, studies.....	387, 589		
stored, effect on formation of serum layer in cream.....	437-488		

	Page		Page
Oxidation-reduction potentials and the hydrogen-ion concentration of a soil. L. G. Willis.....	571-575	Potassium iodide, effect on calves, comparison with iodine.....	112, 119
Parasite, tachinid, of the gipsy moth, <i>Sturmia inconspicua</i> Meigen. R. T. Webber.....	193-208	Potato—	
Parasites, nemtic, and associates of mountain pine beetle (<i>Dendroctonus monticolae</i>). G. Steiner.....	437-444	beetle, Colorado, digestive enzymes and influence of arsenicals on their activity. David E. Fink.....	471-482
Paris green, effect on activity of digestive enzymes of Colorado potato beetle.....	479-481	chloroplasts, origin, development, and increase. Winona E. Stone.....	421-435
Parthenogenesis, in <i>Macrocentrus ancyli-vorus</i>	85	leaves, chloroplasts, origin and division.....	424
Pasteurization—		tubers, greening, effect of light.....	423-424
cream, effect on formation of serum layer.....	480-490, 499	Potatoes—	
milk, effect on serum layer of cream.....	488-489, 499	frozen and unfrozen—	
Pasture grass—		respiration rates.....	547-548, 550-551, 553-554, 555
composition, seasonal variations.....	632-639, 640	sugar-content changes.....	552-553, 554-555
fertilizing and grazing, effect on composition.....	630-632	in storage, physiological studies of. R. C. Wright.....	543 555
Peaches, reaction to carbon dioxide gas, study.....	450-452, 455-458	internal atmospheric composition at different temperatures.....	543, 552, 554
Peas—		sugar content, relation to changes in temperature.....	544-547, 549-550, 552-553, 554-555
chemical composition, changes during storage.....	362-370	Protein content, hard red spring wheats, correlational and allied studies. L. R. Weidron and C. E. Mangels.....	209 231
flavor, preservation in storage, experiments.....	361-370	Puccinia—	
green, shelled—		coronata—	
deterioration in storage.....	361-370	heterothallism, cytological study. Ruth F. Allen.....	513-540
held a few days in storage prior to canning, deterioration. Z. I. Kertesz and E. L. Green.....	361 370	infection, unfertilized and fertilized, study.....	523 540
reaction to carbon dioxide gas, study.....	450-453, 456-458	graminis secalis—	
respiration, effect on deterioration.....	368-370	pathogenicity and prevalence.....	311-312, 314
<i>Pectinophora gossypiella</i> , detection in cotton-seeds by X ray.....	347-348	physiologic forms, distribution and behavior.....	302-306, 308-310, 311-312, 314
Pedigrees, livestock, inbreeding and relationship coefficients, empirical test of the approximate method of calculating. Jay L. Lush.....	565-569	physiologic forms, identification key.....	310
Penicillium stains, of lumber and logs, study.....	233-237	physiologic specialization in. Ralph C. Cotter and Moses N. Levine.....	297-315
Pennsylvania, central, livestock, value of iodine for. E. B. Forbes, Geo. M. Karns, S. I. Bechdel, P. S. Williams, T. B. Keith, E. W. Callenbach, and R. R. Murphy.....	111 128	reactions of host, classification.....	300 316
Phaseolus—		Pugh, Grace Wineland, Johann, Helen, and Dickson, James C.: Relation of the Semipermeable Membranes of the Wheat Kernel to Infection by <i>Gibberella saubinetii</i>	609-626
spp., classification and description.....	25-49	Quince, tissue, migration of <i>Bacillus amylovorus</i> in. Herbert A. Wahl.....	50-64
vulgaris, varieties of field beans, classification. F. H. Steinmetz and A. C. Arny.....	1-50	Rations—	
PHILLIPS, E. F.: Definitions of Honey Color Grades.....	757-770	cattle, net energy value, study.....	186 188, 190
Phosphorus—		dairy cows, influence on chemical and physical properties of milk f.t. O. R. Overman and O. F. Garrett.....	51-58
diet balances for heifers, digestibility determinations.....	558, 560-562	digestibility at different levels of feeding.....	172-173
effect on—		Rhizoctonia—	
efficiency of nitrogen in corn production.....	694-699, 703-704	bottom rot and head rot of cabbage. F. L. Wellman.....	461-469
efficiency of potash in corn production.....	695-697, 700, 702	cabbage, disease production, relation of environment.....	467 468, 469
yield of corn, experiments.....	676-704	rots, of cabbage, causal organism.....	464 465
<i>Phyllosticta rosarum</i> . See <i>Sphaceloma rosarum</i> .		rot, infestation of cabbage, study.....	461-469
<i>Phymatrichum omnivorum</i> —		strains, physiologic specialization.....	465-468
distribution in soil and in plant tissues in relation to control by disinfectants.....	725-739	ROSENBERG, H. A.: Heterothallism and Hybridization in <i>Sphacelotheca sorghi</i> and <i>S. cruenta</i>	287-296
sclerotia—		Roots, asparagus, size, effect on yield.....	102-109
distribution in infested areas.....	731-738, 739	Rose—	
viability and propagating powers.....	730-731, 739	anthracnose—	
Pine—		caused by <i>Sphaceloma</i> . Anna E. Jenkins.....	321-337
mountain, beetle (<i>Dendroctonus monticolae</i>) nemtic parasites and associates. G. Steiner.....	437-444	control measures.....	334-335
southern, lumber and logs, some minor stains. T. C. Scheffer and R. M. Lindgren.....	233-237	name, history, range, and importance.....	323-326
Poisoning chickens, with lead arsenate. E. F. Thomas and A. L. Shealy.....	317-319	inoculation, with <i>Sphaceloma rosarum</i> , experiment.....	334
Potash, effect on—		species and varieties susceptible to anthracnose.....	321-323
efficiency of—		Roses—	
nitrogen in corn production, comparisons.....	689-692, 703-704	leafspot, symptom of anthracnose.....	326-327
phosphorus in corn production, comparisons.....	692-694, 703-704	stem canker, symptom of anthracnose.....	327-328
yield of corn, experiments.....	676-704	Run-off and soil erosion, effect of degree of slope on. F. L. Duley and O. E. Hays.....	349-360
		Rye—	
		inoculation with stem rust, physiologic studies.....	208-314
		stem rust, physiologic specialization in.....	297-315
		Salts, effect on formation of serum layer in cream, experiments.....	485, 490-497, 499

	Page		Page
SCHEFFER, T. C., and LINDGREN, R. M.: Some Minor Stains of Southern Pine and Hardwood Lumber and Logs.....	233-237	SWETT, W. W.; MILLER, FRED W.—Con. GRAVES, R. R.; and C'ERCH, G. T.: Quality, Size, Capacity, Gross Anat- omy, and Histology of Cow Udders in Relation to Milk Production.....	577-607
SCHUMACHER, FRANCIS X., and BULL, HENRY: Determination of the Errors of Estimate of a Forest Survey, with Special Reference to the Bottom-Land Hardwood Forest Region.....	741-756	Swine, feeding with iodine, experiments.....	112-113, 121-122, 127
Serum, separation from bottled cream, study of factors. G. Malcolm Trout and J. C. McComb.....	483-500	Tachinid parasite of the gipsy moth, <i>Stur- mia inconspicua</i> Meigen. R. T. Webber.....	193-208
SEWELL, M. C., and GAINES, P. L.: The Role of Nitrogen in the Production of Spots in Wheat Fields.....	129-148	Tannic acid, effect on growth of <i>Gloosporium perennans</i> and <i>Neofabraea malicorticis</i>	68-71, 70
SHEALY, A. L., and THOMAS, E. F.: Lead Arsenate Poisoning in Chickens.....	317-319	Temperature— effect on.....	543-555
Sheep, inbreeding coefficients, calculation from pedigrees, test of method.....	565-569	growth of <i>Neofabraea malicorticis</i> and <i>Gloosporium perennans</i> , study.....	66, 72-75
Smut fungi, evidences of heterothallism and hybridization in, experiments.....	287-295	Rhizoctonia diseases of cabbage.....	467-468, 469
Soil— erosiveness, study.....	357-358, 360	THOMAS, E. F., and SHEALY, A. L.: Lead Arsenate Poisoning in Chickens.....	317-319
hydrogen ion concentration and oxida- tion reduction potentials. L. G. Willis.....	571-575	THOMPSON, L. G., Jr.: Nitrogen Changes Produced in Certain Nitrogenous Com- pounds by Azotobacter and the Nitrogen Fixed in the Presence of These Com- pounds.....	149-161
liming, effect on oxidation-reduction potential, data and discussion.....	571-574, 575	<i>Tilletia tritici</i> , bunt, resistance to, inheri- tance in hybrids of White Federation and Odessa wheat. Fred N. Briggs.....	501-505
nitrogen, in wheat fields, studies.....	129-148	<i>Trichoderma</i> sp., staining of wood, study.....	235, 237
oxidation reduction potentials and hy- drogen-ion concentration. L. G. Willis.....	571-575	Trotter, G. M., McCOMB, and McCAN, J. C.: A Study of Several Factors in the Separation of Serum from Bottled Cream.....	483-500
Soils, loam, silty clay and sandy, run-off and erosion, comparisons.....	354-358	Tung-oil tree bacterial diseases, Lucia McCulloch and J. B. Demaree.....	339-348
Sphaerocytia cause of rose anthracnose Anna E. Jenkins.....	321-337	leaf, inoculation with <i>Bacterium atropur- pallidum</i> , n. sp.....	341-343
<i>taenium</i> , n. comb. associated fungi and pathogenicity.....	333-334	Vaccination, cows, effects on blood stream, experiments.....	711-724
isolations and cultural characteristics morphology, classification, and identifi- cation.....	332-333	Vaccines, efficacy against infectious abortion of cattle.....	705-724
<i>Sphaerocytia</i> <i>curvata</i> , heterothallism and hybridiza- tion in H. A. Rodenhiser.....	287-296	Vegetables, carbohydrate transformation, effect of carbon dioxide content of storage atmosphere on. Erston V. Miller and Charles Brooks.....	449-459
<i>sorghii</i> , heterothallism and hybridization in H. A. Rodenhiser.....	287-296	Vitamin A, B, C, and G content, Concord grapes, Esther Peterson Daniel and Hazel E. Munsell.....	445-448
Spots, in wheat fields, rôle of nitrogen in production. P. L. Gaines and M. C. Sewell.....	129-148	WALL, HERBERT A.: The Migration of <i>Bacillus anthracis</i> in the Tissue of the Quince.....	50-64
Steers energy metabolism, experiments fasting, relation to heat production, ex- periments.....	175-184 180-184, 189	WAITE, WILLIS W., and FENTON, F. A.: De- tecting Pink Bollworm in Cottonseeds by the X Ray.....	347-348
STEINER, G.: Some Nemic Parasites and Associates of the Mountain Pine Beetle (<i>Pendroctonus manticolae</i>).....	437-444	WALDRON, L. R., and MANGELS, C. E.: Correlational and Allied Studies of the Protein Content, Water Absorption, Loaf Volume, and Loaf Weight of Two Series of Hard Red Spring Wheats.....	209-231
STEINMETZ, F. H., and ARNY, A. C.: A Classification of the Varieties of Field Beans, <i>Phaseolus vulgaris</i>	1-50	WEBBER, R. T.: <i>Sturmia inconspicua</i> Meigen, a Tachinid Parasite of the Gipsy Moth.....	193-208
STONE, WINONA E.: The Origin, Develop- ment, and Increase of Chloroplasts in the Potato.....	421, 435	WELLMAN, F. L.: Rhizoctonia Bottom Rot and Head Rot of Cabbage.....	461-469
Storage— atmosphere, carbon dioxide content, effect on carbohydrate transformation in cer- tain fruits and vegetables. Erston V. Miller and Charles Brooks.....	449-459	Wheat— fields, spots, rôle of nitrogen in produc- tion. P. L. Gaines and M. C. Sewell hybrids, inheritance of resistance to bunt.....	501-505
of potatoes, physiological studies. R. C. Wright.....	543-555	kernel— resistance to <i>Gibberella saubinetii</i>	620-622, 625
<i>Sturmia inconspicua</i> — life and seasonal history.....	194-204, 207-208	semipermeable membranes, relation to <i>Gibberella saubinetii</i> infection. Grace Wineland Pugh, Helen Johann, and James G. Dickson.....	609-626
Meigen, a tachinid parasite of the gipsy moth. R. T. Webber.....	193-208	kernels, structure, description.....	612-618
parasitism and list of hosts.....	204-207, 208	production, relation of nitrogen to, studies.....	129-148
synonymy, distribution, and importation.....	193-194	resistance to bunt, <i>Tilletia tritici</i> , inheri- tance in hybrids of White Federation and Odessa. Fred N. Briggs.....	501-505
STURTEVANT, A. P.: Relation of Commer- cial Honey to the Spread of American Foulbrood.....	257-285	scab— fungus, entrance and spread in wheat kernels, factors.....	624, 625
SWETT, W. W.; MILLER, FRED W.—Con. and GRAVES, R. R.:— Comparison of Conformation, Anat- omy, and Skeletal Structure of the Cow and Bull of a Dairy Breed.....	641-674	See also <i>Gibberella saubinetii</i> .	
Composition of Milk Obtained from Amputated Cow Udders.....	401-419		
Quantity of Milk Obtained from Am- putated Cow Udders.....	385-400		

	Page		Page
Wheat—Continued.		WILLIAMS, P. S.; FORBES, E. B.; KARNs, GEO. M.; BECHDEL, S. I.; KEITH, T. B.; CALLENRACH, E. W.; and MURPHY, R. R.: The Value of Iodine for Livestock in Central Pennsylvania.....	111-128
White Federation and Odessa hybrids, inheritance of resistance to bunt, <i>Tilletia tritici</i> . Fred N. Briggs.....	501-505	WILLIS, L. G.: Oxidation-Reduction Potentials and the Hydrogen-Ion Concentration of a Soil.....	571-575
yield, nitrogen-treated soil, experiments. 142-148		Wood—	
Wheatgrass, crested—		oil tree. See Tung-oil tree.	
inflorescence, development and description.....	382	stains, caused by treatment for blue stain, study.....	233-237
life history and habits. L. Dudley Love and Herbert C. Hanson.....	371-383	WRIGHT, R. C.: Some Physiological Studies of Potatoes in Storage.....	543-555
mature plant, development and morphology.....	377-382		
seed, description and germination.....	371-372	X Ray, detection of pink bollworms in cottonseeds. F. A. Fenton and Willis W. Waite.....	347-348
seedling, development and morphology.....	373-377		
Wheats, hard red spring, two series, correlational and allied studies of the protein content, water absorption, loaf volume, and loaf weight. L. R. Waldron and C. E. Mangels.....	209-231		

Indian Agricultural Research Institute (Pusa)
LIBRARY, NEW DELHI-110012

This book can be issued on or before

Return Date	Return Date